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THE PENETRATION OF THE INSECT CUTICLE BY ISOMERS OF BENZENE HEXACHLORIDE

BY G. ARMSTRONG, F. R. BRADBURY AND H. STANDEN

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(With 1 Text-figure)

The penetration of the α-, β-, γ- and δ-isomers of benzene hexachloride through the insect cuticle has been studied by a micro-colorimetric technique.

Grain weevils were exposed for varying periods to deposits of pure isomers of benzene hexachloride on filter-paper. The amounts of each isomer picked up by the insects were determined as two fractions, one 'outside' representing that which could be removed by cold methanol washing, and the other 'inside' recovered after decomposing the insects with nitric acid.

The results show that the amounts of each isomer taken up by the insects are in the approximate ratio of their solubilities in hydrocarbon solvents, and that the γ-isomer penetrates through the outer layers of the insect integument much more rapidly than the other three isomers tested.

On the basis of these results it is concluded: (i) that the first stage of pick-up of insecticide by the insects is simple solution of the insecticide in the outer waxy covering of the epicuticle; and (ii) that structural effects play an important part in the penetration through the insect cuticle as well as in toxic effects at the site of action.

INTRODUCTION

It is generally accepted that an active 'contact' insecticide must possess two essential properties. It must be toxic to the insect and it must be capable of gaining access to the site of its action by penetration through the insect cuticle.

For the study of penetration of the insect integument the isomers of benzene hexachloride, $C_6H_6Cl_6$, present an interesting series of compounds in that insecticidal activity is shown by the γ-isomer only. It is of interest to inquire whether this specific structural effect shows itself in toxic action 'inside' the insect, or in ability to penetrate the insect cuticle, or in both processes. Dresden & Krijgsman (1948) have approached this problem by an injection technique, and shown that of the four isomers, α, β, γ and δ, only γ is toxic to the American cockroach, *Periplaneta americana*, when injected into the body cavity. They conclude from this that the greater toxicity of the γ-isomer is not a consequence of quicker penetration, but due to the stereo-configuration of the γ-molecule which allows a specific attack on an unknown physiological process. Whilst it is probably true that the toxic action of γ-benzene hexachloride is a function of its stereo-configuration, it does not follow from Krijgsman's work that stereo-isomerism in benzene hexachloride is unrelated to the question of penetrating the insect cuticle.

The work to be described attempts to answer the question 'Do the four isomers of benzene hexachloride penetrate the insect cuticle equally readily?' and also presents some quantitative information on the rate of pick-up of benzene hexachloride from a deposit by insects kept in contact with it.

Method

The experimental work is based on a colorimetric method for determining small quantities of benzene hexachloride in the presence of organic matter. The process, which was developed by Mr H. K. Southern and Mr A. Williams in the Analytical Department of this laboratory, is fully described in the experimental part. It depends on dehydrohalogenation of benzene hexachloride followed by nitration. The resulting nitrohalobenzenes give with alkali a yellow colour which is measured in the Hilger 'Spekker' absorptiometer. The method does not distinguish between the four isomers of benzene hexachlorides, each of which gives the same colour reaction, but the amounts of the individual isomers recovered differ and it is, therefore, necessary to calibrate the instrument separately for each isomer. The authors of the method claim that it is capable of determining down to 50μg. of benzene hexachloride, but we obtain measurable colour with as little as 10μg. The accuracy of the analysis varies with the procedure employed, and is discussed under 'Accuracy of the Method'.

The main experiments have been done with the grain weevil *Calandra granaria*. These were exposed to deposits of the pure isomers on filter-paper for varying periods. The benzene hexachloride picked up was estimated as two fractions. The first fraction was recovered from the insects either by steam distilling them or by repeated washings with cold methanol. The second fraction was obtained by decomposing the insects with hot concentrated nitric acid on the water-bath, and subsequently distilling out with steam the benzene hexachloride so liberated.

It was hoped in this way to distinguish between superficial 'pick-up' of insecticide by the insects and penetration through the insect integument.

Some preliminary experiments were done to test this point, grain weevils (2·5 g.) were exposed to filter-papers impregnated with technical γ-benzene hexachloride at 113μg./cm.^{2} for 22 hr. One batch of treated weevils was subjected to steam distillation four times in succession, each distillate being analysed separately for benzene hexachloride. The weevils were then decomposed by nitric acid treatment, and the remaining benzene hexachloride determined. A second batch of treated weevils was given four successive methanol washes (one wash being a quick rinse in the cold methanol with 10, 5 and 5 ml.), and each washing separately analysed for benzene hexachloride. The weevils of this batch were then subjected to one steam distillation, and finally decomposed by nitric acid treatment. The figures for benzene hexachloride found at each stage are set out in Table 1.

The figures show that the bulk of the benzene hexachloride recovered is super-

ficial, being removed by the first steam distillation or the first two methanol washes. On the other hand, even after repeated steam distillation or methanol washing, a substantial amount remains to be recovered after decomposing the insects by nitric acid treatment. Repeated steam distillation appears to cause loss of benzene hexachloride, as shown by a comparison of the totals for the two methods of treatment. For the main part of the work, therefore, we standardized on two methanol washes for removal of insecticide from superficial layers, followed by nitric acid decomposition to set free that which had penetrated more deeply. The two fractions so determined are referred to throughout the paper as 'outside' and 'inside' fractions.

TABLE 1. *Isolation of γ-benzene hexachloride from treated weevils*

Treatment	Benzene hexachloride found (μg./g.)	Treatment	Benzene hexachloride found (μg./g.)
Steam distillation 1	144	Methanol wash 1	120
Steam distillation 2	15	Methanol wash 2	34
Steam distillation 3	2	Methanol wash 3	8
Steam distillation 4	3	Methanol wash 4	8
		Methanol wash and steam distillation	15
HNO_3 decomposition	12	HNO_3 decomposition	30
Total	176	Total	215

N.B. The technical γ-isomer used in this experiment appears to be taken up by insects much more readily than the pure γ-isomer used in the main part of the work.

While this work was in progress, Sternburg, Kearns & Bruce (1950) published their work on the absorption of DDT by house-flies in which they used a similar technique to distinguish between DDT absorbed by the flies and that which remained on the cuticle. The flies were rinsed twice with diethyl ether to remove superficial DDT, and the absorbed material was liberated by grinding with sodium sulphate followed by extraction with solvent. In support of this method of separation, the authors quote experiments in which DDT was injected into the body cavity of flies. Such flies fail to give up their DDT to the ether rinse, but it was recovered after grinding the flies and extracting. Perry & Hoskins (1950), in similar work on the penetration of DDT into various strains of house-flies, removed 'adhering DDT' by a thorough rinse with chloroform, subsequently extracting internal DDT with chloroform after grinding. We have applied this test to our technique for separating 'outside' material from 'inside' and find that blowflies injected with benzene hexachloride do not give it up to methanol washing, whereas it can be recovered after nitric acid decomposition of the insects. We have preferred methanol to ether or chloroform for removing benzene hexachloride from 'outside' the insects since, according to Beament (1948), it is a poor solvent for the wax of the insect epicuticle. Ether and chloroform, being good solvents for the wax layer, may remove this barrier and so give rise to a new set of equilibrium conditions very different from those obtaining with intact insects.

EXPERIMENTAL PROCEDURE

Analytical method

The method set out below describes the procedure used for determining the benzene hexachloride 'inside' the insects, including the decomposition of the insects with nitric acid. For determination of 'outside' benzene hexachloride the methanol washes are diluted to 450 ml. with water, and then treated as the steam distillate in the procedure set out below, starting from the point: 'Transfer to separating funnel, and extract three times with approximately 4 ml. portions of carbon tetrachloride....'

Reagents

Concentrated nitric acid
Concentrated sulphuric acid
Nitration mixture (1 vol. sulphuric + 1 vol. fuming nitric acid)
Sodium hydroxide 10%, w/w solution
Sodium hydroxide solution, sp.gr. 1·4 (36·4%)
Dilute sulphuric acid (concentrated sulphuric acid, 1 vol. + water 3 vol.)
Sodium carbonate solution (0·5% Na_2CO_3 in water)
Ether
Carbon tetrachloride

(All should be of reagent quality)

Apparatus

Flasks: 50 ml. capacity with flat bottoms and A 14 round glass neck and with a tubular projection (nipple) of volume approximately 0·5 ml. sealed half-way up side. Steam generator, 3 l. capacity; distillation flask, 1 l. capacity; separating funnels, 50, 100 and 500 ml.; condenser, 2 ft. water jacketed with A 14 joints.

Procedure

Transfer the washed insects to a 1 l. round-bottom flask and add 80 ml. of concentrated nitric acid, place on the steam-bath and heat until the insects are completely decomposed. Dilute with water to 600 ml. and connect the flask to a steam generator, and distil 300 ml. by heating the flask and blowing steam into the mixture. To the distillate add gradually 70 ml. concentrated sulphuric acid cooling thoroughly. Transfer to a separating funnel, and extract three times with approximately 4 ml. portions of carbon tetrachloride. Combine the extracts and transfer to a 50 ml. flask. Partially immerse the flask in a beaker of hot water and evaporate by blowing a gentle stream of air (dried and cleaned by passing through a caustic soda tower) to a volume of about 3 ml. Remove the flask from the beaker of water and continue the evaporation by blowing over the surface at room temperature until the projection on the flask is just filled, giving a volume of approximately 0·5 ml. Add 4 ml. concentrated sulphuric acid-fuming nitric acid mixture and heat under reflux by attaching the 2 ft. water-cooled condenser. Benzene hexachloride is stable to this procedure. After 30 min., cool the flask and add about 20 ml. of water through the condenser. Boil for 15 min. in order to remove oxides of nitrogen, cool, wash down the condenser into the flask and transfer the contents to a separating funnel, diluting to a total volume of about 80 ml. Add sodium hydroxide solution (sp.gr. 1·4) slowly, whilst cooling until the contents are distinctly alkaline to litmus paper. About 9 ml. of sodium hydroxide solution (sp.gr. 1·4) will be required, and up to 0·5 ml. in excess may be added. Extract the solution quickly with three 3 ml. portions of a mixture of 8 ml. ether + 1 ml. carbon tetrachloride. Wash the extracts twice with 1 ml. quantities of water and transfer the extracts to a 50 ml. flask, pouring the ether through the top of the separating funnel. Wash out the funnel with up to 2 ml. of ether, adding this to the flask. Reduce the contents of the flask to a final volume of 0·5 ml. by

means of a current of dry air. Add 20–25 ml. water and 20 ml. sodium hydroxide solution (10%), insert a boiling rod (to prevent bumping), connect the flask to the 2 ft. water-cooled condenser and boil under reflux for 1 hr. Cool the flask, wash down the condenser and extract the solution three times with a total of 5 ml. of carbon tetrachloride. Wash the combined extracts twice with 2 ml. quantities of water and transfer through the dried stem of a separating funnel to a 50 ml. flask, and evaporate to 0·5 ml. in a stream of dry air. Add 4 ml. of sulphuric acid-fuming nitric acid mixture and place the flask on the metal surface of a steam-bath for 45 min. in order to effect nitration. Connect the flask to a 2 ft. condenser after inserting a boiling rod, dilute the contents of the flask to about 25 ml. by pouring water through the condenser and boil under reflux for about 15 min. Remove the bunsen flame and pour carefully and slowly down the condenser 12 ml. sodium hydroxide solution (sp.gr. 1·4). Reflux the alkaline solution for 15 min. in order to complete the development of the yellow colour. Cool the flask, wash down the condenser and transfer the contents of the flask to a separating funnel; add 20 ml. dilute sulphuric acid in order to make the solution acid. Extract the solution three times with small quantities of ether. Wash the ethereal extracts twice with 1 ml. quantities of water and then extract the sodium salt of the nitro body twice with 0·5% sodium carbonate solution. Make up the volume of the combined extracts to 10 ml. with distilled water, and determine the amount of benzene hexachloride either by comparison with standard colours prepared from known amounts of benzene hexachloride or by use of the Spekker absorptiometer using 1 cm. cells and no. 601 dark blue Ilford filters.

Precautions

In all determinations the following precautions should be taken:

(1) No grease should be employed on taps and stoppers of separating funnels, etc.

(2) The same 50 ml. flask should be used throughout a determination, and before a solvent is put into it for evaporation it should be washed with water, alcohol and then ether and allowed to remain inverted for a few minutes.

(3) The solvent aliquots used for extraction should be first put into the flask from which the aqueous solution has been taken before adding to the main solution in the separating funnel, and great care must be taken to ensure that the small amounts of solvent in which nitration and hydrolysis, etc., are carried out, are not lost.

(4) If at any time during evaporations the solvent is completely lost some of the benzene hexachloride or trichlorobenzene may also be lost, and the determination should be discontinued and repeated on a fresh portion of sample.

Calibration

When the whole insects were decomposed with nitric acid and steam distilled, the amount of benzene hexachloride recovered was less than when the determinations were made on methanol washes. Calibrations by the procedure described below were made for each isomer, one for 'outside' determinations and one for 'inside'. The 'outside' points for α-, β-, γ- and δ-isomers were found not to differ significantly, and they were combined to give one line. Different amounts of the four isomers were recovered after the 'inside' treatment, and separate curves were therefore drawn for each. The procedure for determining calibration curves for 'outside' values is as follows:

> Dissolve 0·05 g. of the pure isomer in 500 ml. of methanol. Wash 2·5 g. of untreated grain weevils with methanol as described under 'Recovery of benzene hexachloride from insects', and to the washings add aliquot portions of the standard solution and proceed with the determination as described above for 'outside' determination.

For calibrating 'inside' measurements the procedure is as follows:

> 2·5 g. untreated grain weevils are washed with methanol as before, and the washed weevils transferred to the 1 l. flask. Aliquot portions of the standard solution of the

benzene hexachloride isomer are added, and the determination of benzene hexachloride made as described above for 'inside' measurements.

In all the calibrations described above, the best fit to the points was a straight line. The five curves are summarized in Table 2 by the regression equations of y on x, y being the Spekker reading and x the amount of benzene hexachloride expressed in micrograms. Inspection of the equations makes it clear that, with the exception of α-isomer, the recovery of benzene hexachloride is always poorer after the nitric acid and steam-distilled treatment than when these processes are avoided in the determinations on methanol washings. It is possible that the loss occurs in the steam distillation where the low volatility of β and the greater solubility of γ and δ may account for the incomplete recovery of these isomers.

TABLE 2. *Calibration curves and limits of error*

Isomer	Treatment	Regression equation of calibration curve	Limits of error: Due to analytical method (reduced by 1/2·5) for single determination (μg.)	Limits of error: Due to total variation (includes biological as well as analytical) for single determination (μg.)
$\alpha, \beta, \gamma, \delta$	Methanol washings ('outside')	$y=0{\cdot}039+0{\cdot}00138x$	$\pm$ 8	$\pm$25·4
α	Nitric acid decomposition and steam distillation ('inside')	$y=0{\cdot}033+0{\cdot}00161x$	$\pm$10	—
β	Nitric acid decomposition and steam distillation ('inside')	$y=0{\cdot}033+0{\cdot}00081x$	$\pm$12	—
γ	Nitric acid decomposition and steam distillation ('inside')	$y=0{\cdot}045+0{\cdot}00115x$	$\pm$12	$\pm$18
δ	Nitric acid decomposition and steam distillation ('inside')	$y=0{\cdot}0365+0{\cdot}00085x$	$\pm$11	$\pm$ 9

Exposure of insects to benzene hexachloride

The procedure for each exposure was as follows:

By means of a pipette, 2 ml. of an acetone solution of the required benzene hexachloride isomer was applied evenly to a 15 cm. Whatman no. 1 filter-paper. To obtain a deposit of 11 μg./cm.^{2}, a 0·1% solution was used. When dry the paper was fitted into the bottom of a 15 cm. Petri dish, and fixed firmly to the glass with 'Sello-tape' to prevent the insects crawling underneath the paper.

2·5 g. of grain weevils were then poured into the dish and the cover fitted on.

To ensure that the insects remained fairly evenly distributed over the surface of the paper, the dish was placed in a drawer, away from the light (in the presence of light the insects would accumulate on the side of the dish farthest away from it). The exposure was done in a constant temperature and humidity room (temperature range 74–80° F., percentage relative humidity 60–72).

An experiment was done to investigate the possibility of the benzene hexachloride deposit available for pick-up becoming exhausted during a long exposure. Two separate 2·5 g. samples of grain weevils were exposed consecutively on the same paper—at a concentration of 11 μg./cm.^{2}—for 72 and 4 hr. The subsequent benzene hexachloride estimation yielded, in the case of the 4 hr. exposure, a similar result to values obtained when a freshly prepared paper was used.

These results show that there is no appreciable decline in the availability of the benzene hexachloride in the deposit during exposures of 76 hr. or less.

Recovery of benzene hexachloride from treated insects

The grain weevils, after exposure to benzene hexachloride, were placed in a 100 ml. pear-shaped separating funnel fitted with a small plug of glass-wool above the tap. Ten ml. of methanol were poured in, and, after gentle shaking for 30 sec., the methanol was run off. Two further washings were given with 5 ml. portions of the solvent and the whole procedure repeated, the total volume of methanol used being 40 ml.

The washings were diluted to 450 ml. with water and extracted three times with a total of 12 ml. of carbon tetrachloride. The extracts were then subjected to the normal benzene hexachloride estimation.

After washing, the 'inside' benzene hexachloride was estimated by placing the washed weevils in a 1 l. flask and decomposing with nitric acid as described in 'Analytical method'.

TABLE 3. *The pick-up and penetration of the isomers of benzene hexachloride into grain weevils*

Exposure (hr.)	Isomer ...	α					β					γ					δ				
	Replicate...	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
½	Outside	—	—	—	—	—	—	—	—	—	—	15	7	—	—	—	15	12	—	—	—
	Inside	—	—	—	—	—	—	—	—	—	—	2	6	—	—	—	8	16	—	—	—
	Total	—	—	—	—	—	—	—	—	—	—	17	13	—	—	—	23	28	—	—	—
1	Outside	—	—	—	—	—	—	—	—	—	—	21	16	—	—	—	25	20	—	—	—
	Inside	—	—	—	—	—	—	—	—	—	—	0	6	—	—	—	11	10	—	—	—
	Total	—	—	—	—	—	—	—	—	—	—	21	22	—	—	—	36	30	—	—	—
4	Outside	13	3	16	—	—	2	1	—	—	—	47	53	—	—	—	48	71	47	49	64
	Inside	8	5	0	—	—	8	0	—	—	—	19	14	—	—	—	10	9	8	18	8
	Total	21	8	16	—	—	10	1	—	—	—	66	67	—	—	—	58	80	55	67	72
7	Outside	—	—	—	—	—	—	—	—	—	—	66	72	42	38	43	102	105	120	—	—
	Inside	—	—	—	—	—	—	—	—	—	—	47	50	22	45	28	10	9	17	—	—
	Total	—	—	—	—	—	—	—	—	—	—	113	122	64	83	71	112	114	137	—	—
12	Outside	—	—	—	—	—	—	—	—	—	—	51	69	51	74	—	72	104	107	—	—
	Inside	—	—	—	—	—	—	—	—	—	—	44	29	52	51	—	8	8	9	—	—
	Total	—	—	—	—	—	—	—	—	—	—	95	98	103	125	—	80	112	116	—	—
22	Outside	6	18	—	—	—	5	1	—	—	—	34	46	28	40	—	66	96	112	—	—
	Inside	5	1	—	—	—	9	0	—	—	—	26	29	28	31	—	25	31	38	—	—
	Total	11	19	—	—	—	14	1	—	—	—	60	75	56	71	—	91	127	150	—	—
48	Outside	7	—	—	—	—	1	—	—	—	—	58	78	—	—	—	98	—	—	—	—
	Inside	6	—	—	—	—	1	—	—	—	—	147	162	—	—	—	54	—	—	—	—
	Total	13	—	—	—	—	2	—	—	—	—	205	240	—	—	—	152	—	—	—	—
65	Outside	—	—	—	—	—	0	—	—	—	—	60	—	—	—	—	—	—	—	—	—
	Inside	—	—	—	—	—	1	—	—	—	—	171	—	—	—	—	—	—	—	—	—
	Total	—	—	—	—	—	1	—	—	—	—	231	—	—	—	—	—	—	—	—	—
72	Outside	7	—	—	—	—	—	—	—	—	—	79	50	60	—	—	88	58	—	—	—
	Inside	34	—	—	—	—	—	—	—	—	—	196	137	155	—	—	48	67	—	—	—
	Total	41	—	—	—	—	—	—	—	—	—	275	187	215	—	—	136	125	—	—	—

RESULTS

Results of experiments are listed in Table 3. The original plan was to test the four isomers α, β, γ and δ of benzene hexachloride as a series of different exposure times ranging from 0·5 to 72 hr. It soon became obvious, however, that the exposure to α- and β-isomers for short periods was pointless, since so little was picked up in the longer exposure times.

In Fig. 1 the amounts of benzene hexachloride 'outside' and 'inside' are plotted as μg./g. of insect against the time of exposure to the deposit, to give a picture of the process of pick-up and absorption of the isomers.

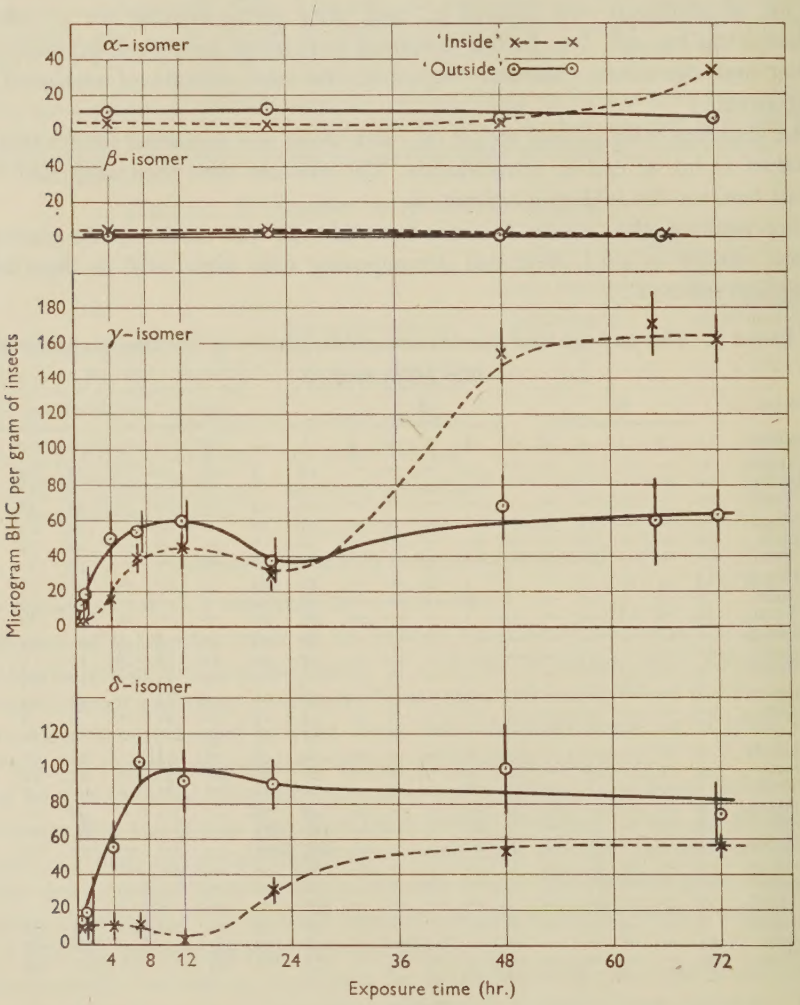

Fig. 1. Penetration of benzene hexachloride isomers into grain weevils.

Accuracy of the method

From the points of the calibration curves, it is possible to calculate the limits of error of the analytical method. The 5% probability limits of error for single determinations for 'outside' figures are $\pm 20\mu$g.; and for 'inside': $\alpha \pm 24$, $\beta \pm 30$,

$\gamma \pm 30$ and $\delta \pm 27 \mu$g. For the figures in Table 2, where determinations on 2·5 g. of weevils have been reduced to μg./g. of insects, the limits are correspondingly reduced to the figures shown in column 4 of Table 2. These limits refer only to the error of the analytical method; a further source of error not taken into account in these figures is the variation in the biological material. The limits of error calculated from the 'among replicates' variance, using the figures in Table 3 are given in the end column of Table 2. These refer to limits for single determinations, and can be converted to limits for mean values by dividing by $\sqrt{n}$, where n is the number of determinations. The points in Fig. 1 are in most cases the mean of two or more determinations, and the probable limits of error are expressed by the vertical line passing through or near each point. It is evident that the overall errors are not great enough to invalidate the general shape of the curves.

Estimation of the volume of wax in the waxy layer of the epicuticle of grain weevils

Grain weevils (15 g.) were rolled with γ-alumina for 2 hr. The bulk of the alumina was separated from the insects by sieving, and extracted three times with 20 ml. volumes of boiling chloroform. The insects, with some adhering alumina, were extracted three times with 20 ml. volumes of cold chloroform. The combined filtered extracts were freed from solvent, leaving 30·8 mg. of a brown amorphous solid. This solid was extracted three times with boiling acetone and the hot solutions filtered and concentrated to approximately 3 ml. When this concentrated solution was cooled to 0° C. flocculent masses of crystals were deposited. These were isolated and weighed, yielding 9·2 mg. A further extraction of the brown solid with boiling acetone gave 1·1 mg. more of the crystalline material. If the grain weevil be regarded as a cylinder 2 mm. long by 1 mm. diameter, the number per g. between 500 and 600 and the density of the wax as 1·0 this weight corresponds to a wax layer between 0·1 and 0·2μ thick. Beament (1945) has found wax-layer thicknesses ranging from 0·095 to 0·6μ for various insects.

Discussion

The first point of interest in these results is the relative amount picked up by the insects from deposits of the four isomers of equal density. If the pick-up had been no more than superficial contamination, it might have been expected that the amounts of the four isomers recovered as 'outside' would have been equal. This is clearly not the case. The amounts of each isomer found 'outside' the insects are in the approximate ratio of the solubilities of the isomers in hydrocarbons, esters and ether. At the peaks of the curves (7–12 hr. exposure) the 'outside' amounts of benzene hexachloride recovered for the four isomers are α 12, β 3, γ 60 and δ 102 μg./g. of insect, which can be compared with the solubilities in diethyl ether at 20° C. which are α 6·6, β 1·8, γ 26·3, δ 54·9 (g./100 g. solvent). This suggests that

what is removed by the methanol wash is in solution in the outer layer of the insect integument, possibly the outer waxy layer of the epicuticle.

Wigglesworth (1945) and Beament (1945) have described this outer layer of wax for a number of insects, and given estimates of its thickness. To estimate the amount of wax in this layer of the grain weevil epicuticle we followed closely the technique described by Beament, with the addition of a preliminary treatment of the insects with alumina to abrade the protective cement layer (Wigglesworth, 1945) before extracting the wax with chloroform. By this treatment, which is described in detail in the 'Experimental part', we found the weight of wax per gram of insects to be $6{\cdot}9 \times 10^{-4}$ g. If the maximum amounts of isomers found 'outside' (after 7–12 hr. exposure) are dissolved in this weight of wax their solubilities stand in the ratios set out in Table 4.

Table 4. *Calculated 'solubilities' of the benzene hexachloride isomers in insect wax compared with solubilities in petroleum* (*g.* 100 *g. solvent*)

	Isomer			
Solvent	α	β	γ	δ
Insect wax	1·7	0·4	8·7	14·8
Petroleum oil, Grade 5*	1·5	0·7	5·2	13·4

* A mineral oil used for spraying fruit trees; its specification requires not more than 10 % to boil below 320° C. and not more than 55 % below 360° C.

While the figures for solubility in insect wax are no more than very rough approximations, their order and ratio bear a striking resemblance to the figures for petroleum oil. The similarity is interesting, in view of evidence by Bergmann (1938) and Chibnall and co-workers (1934) that the wax of the insect epicuticle consists of mixtures of paraffins, alcohols and fatty acids.

The second interesting point is the difference between the γ-isomer and the others with regard to the shape of the 'inside' curve. Up to 12 hr. the γ-isomer is the only one which shows substantial amounts 'inside' the insect and it appears, therefore, that the γ-isomer penetrates the insect cuticle more readily than the other three isomers. It is only after 22 hr. exposure that the δ-isomer begins to appear in substantial amounts 'inside' the insect, despite the fact that its 'outside' concentration is greater than that of γ at all points in the curve. It therefore appears that the structural difference between the isomers of benzene hexachloride have a considerable effect on the ability of the isomers to penetrate the insect cuticle. This is an important modification of the conclusion drawn by Dresden & Krijgsman (1948), who claimed that structural effects relate only to the toxic action 'inside' the insect and not to the question of penetration.

It may be that the developing toxic effects of the small amount of γ-isomer which penetrates in the first few hours brings in its train an increase in the permeability to benzene hexachloride of the epicuticle, and that the rapid penetration of the

insect cuticle is a secondary effect depending on the toxic action of this isomer. Even after only half an hour's exposure to γ-isomer sufficient poison penetrates to produce almost 100% kill of the insects. This is illustrated in Table 5, where the mortalities at varying periods after removal from the treated surface are recorded for insects exposed to γ- and δ-isomers.

The idea that mass penetration by γ-isomer is a secondary effect following the establishment of toxic symptoms receives some support from the amounts of γ-benzene hexachloride discovered 'inside' the insects after 48 hr. or longer exposure to the deposit. At 27 hr. the 'inside' curve crosses the 'outside' and thereafter increases rapidly, so that after 72 hr. exposure about 160 μg. of γ-isomer per gram of insect has penetrated, while the 'outside' concentration has slowly increased to a value approximately equal to the peak value at 8–12 hr. It is evident from Table 5 that after 22 hr. exposure almost all the insects are dead, and it appears that the γ-benzene hexachloride penetrates the dead insects freely and builds up 'inside' to the high values found at 48 and 72 hr.

TABLE 5. *Toxicity of deposits of γ- and δ-isomers to grain weevils*

Isomer	Exposed to paper containing 11 μg./cm.^{2} (hours)	Percentage kills (hours after removal from paper)			
		0	24	48	72
γ	$\frac{1}{2}$	0	53	85	94
	1	0	48	90	97
	4	0	92	95	97
	7	50	81	97	—
	12	80	89	—	—
	22	84	96	100	—
δ	22	0	0	0	0

Note. Percentage kills are means of four batches of fifty insects.

The existence of a distinct peak in both the 'outside' and 'inside' γ-curves cannot be readily explained, but it is possible that the fall in the concentration 'outside' is connected with the immobilization of the insects resulting from the toxic effect of the γ-isomer which becomes pronounced after 7 hr. exposure. In the earlier stages of exposure the insects move about the paper freely, but after 7 hr. or longer exposure at least half the insects are incapable of walking and many of them are dead. This is shown clearly in Table 5.

If as a result of immobilization of the insect the rate of pick-up of γ-benzene hexachloride is reduced but penetration from 'outside' to 'inside' continues as before, it might be expected that the γ-benzene hexachloride found 'inside' would rise so that the total 'outside' and 'inside' remained constant. The fact that this is not so suggests that the insect removes some of the material which has penetrated. If this is metabolized, say to trichlorobenzene, it would not appear as benzene hexachloride in our analysis, since it would be converted to a nitrohalobenzene in

the first nitration and then extracted by the sodium hydroxide treatment which follows this.

The results expressed in the curves of Fig. 1 may be briefly interpreted as follows. When insects remain in contact with filter-paper impregnated with benzene hexachloride the isomers each slowly dissolve, probably in the waxy outer layer of the epicuticle, and after 7 hr. exposure the amounts dissolved are roughly proportional to the solubilities of each isomer. In the case of the γ-isomer only there is also extensive penetration during the first 7 hr. into regions below the outer layers. This results in toxic symptoms, 50% of the insects being apparently dead after 7 hr. exposure and 84% after 22 hr. (see Table 5). Those which are still alive after 7 hr. are relatively immobile. In the period 12–22 hr. the amount of γ-isomer found 'outside' the insects shows a fall, and it is possible that the rate of solution in the wax layer is reduced owing to the relative immobility of the insects, whilst the rate of penetration remains constant or increases. The fact that the 'inside' curve does not show an increase until all, or almost all, the insects are dead—after 22 hr. exposure—and then shows a rapid build-up cannot be explained at present.

The δ-isomer is the only one, other than γ, to show any considerable degree of penetration of the insect epicuticle, but since it exerts no toxic effects on the insect the equilibrium condition reached after 24 hr. exposure is not much changed at 48 and 72 hr.

Work is continuing on the problem of interpreting the shape of the curves and the mechanism of penetration of insecticides through the insect integument.

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PARTICLE SIZE OF INSECTICIDAL SUSPENSIONS AND THEIR CONTACT TOXICITY

III. TEMPERATURE COEFFICIENTS AND TESTS BY INJECTION

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(With 4 Text-figures)

Aqueous DDT suspensions containing particles of different sizes gave different temperature coefficients of mortality in dipping tests against adult *Oryzaephilus surinamensis* and *Tribolium castaneum*. In the range 12–30° C., colloidal DDT showed a large negative coefficient, but the coefficient for a suspension of DDT crystals of 400μ was smaller (*T. castaneum*) or negligible (*O. surinamensis*). Thus, although a suspension of crystalline DDT was more toxic than colloidal DDT to warm insects, it was less toxic than the colloid to cool insects.

A test was made of rotenone suspensions against adult milkweed bugs (*Oncopeltus fasciatus* Dall.) by application of measured droplets under the wings. Colloidal rotenone was more toxic than a crystalline suspension. Milkweed bugs could not be killed in this way by DDT suspensions.

Tests were also made by injecting suspensions of rotenone, DDT and its fluorine analogue (DFDT) into milkweed bugs. A suspension of rotenone crystals was equitoxic with colloidal rotenone against insects kept for 2 days at 27° C. after treatment. The same was true of crystalline and colloidal DDT suspensions. If the bugs were kept at 10° C., colloidal rotenone was much more toxic than crystalline rotenone 2 days after treatment. But if the insects were kept for about 3 weeks and inspected at intervals, the first apparent difference in toxicity grew smaller, and in the end it nearly disappeared. With DDT the time for this to occur was about 10 days; and suspensions of colloidal and crystalline DFDT were equitoxic after only 2 days, even at 10° C.

In the application test, the difference between colloidal and crystalline rotenone was a real difference in toxicity. It did not disappear with the lapse of time; but in the injection tests the two forms of each poison differed only in speed of action, and not in ultimate toxicity.

Introduction

Parts I and II of this series (McIntosh, 1947*b*, 1949) described dipping and spraying tests of simple aqueous suspensions of pure DDT and rotenone against grain beetles. For convenience, these papers are referred to below simply as 'Part I' and 'Part II'.

From Part I it is plain that DDT from large crystals (400μ) can easily penetrate the cuticle of grain beetles. Smaller particles are not so efficiently retained on the surface of the insects but, once in position, are no more effective than large ones, weight for weight. Van den Hende & Jacobs (1950) have tested the types of suspension

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used in Part I as direct sprays and as films on glass and filter-paper against *Tribolium confusum*, with similar results.

On the other hand, Part II showed that toxicity of rotenone suspensions is inversely related to particle size, in spite of the low retention of small particles. The effects of time on kill were also measured; both colloidal and crystalline DDT gave kills which increased as time went on. Crystalline rotenone behaved in the same way, but colloidal rotenone gave what seemed to be immediate paralysis, followed by recovery of some of the insects.

This paper gives an account of further tests, which are mainly on the effect of temperature on contact toxicity and on toxicity by injection. They were made to show the importance of lipoid solubility in deciding the relative toxicities of different sized poison particles; their results seem to confirm a suggested explanation of the results of Parts I and II. This explanation, along with the theory of the present tests, is given in Part IV of this series (McIntosh, 1951).

EFFECTS OF TEMPERATURE ON CONTACT TOXICITY OF DDT SUSPENSIONS

In the earlier tests, the insects were always kept at constant temperatures after treatment. This section describes tests in which the after-temperatures were varied.

Adult *Oryzaephilus surinamensis* were treated with DDT needle aggregates and colloid by the dipping method (McIntosh, 1947*a*, and Part II). At each concentration, thirty insects were immersed in 8 ml. suspension for $1\frac{1}{4}$ min. at 27° C.; they were afterwards kept at either 30° C. and 35% R.H., or 12° C. and 65% R.H., without food, and were inspected after 24 hr.

The results are given as probit-log concentration lines in Fig. 1, graph A, calculated for best fit. The symbols ↑ and ↓ show concentrations which gave kills of 100 and 0% respectively. Some details of the probit analyses and size measurements are given in Table 1. The equations take the form $y = bx + c$, where y = probit kill, b = gradient of line, x = log (percentage concentration $\times 10^4$) and c = constant. The values of x when $y = 5$ [i.e. log (median lethal concentration $\times 10^4$) or log (M.L.C. $\times 10^4$)] were calculated from the equations, and their ratios are given in the last column; in Table 1, the M.L.C. for colloid was always used as numerator.

At 30° C., needle aggregates are 5·4 times more toxic than colloid, but at 12° C. the ratio is reversed, the colloid being 7·9 times more toxic than the aggregates. Needle aggregates show a very small temperature coefficient of mortality (1·8 times over 18°), but with colloidal DDT there is obviously a very big negative coefficient (78 times over 18°), so that the small particles are the more toxic at the lower temperature.

Similar tests were made on adult *Tribolium castaneum* by the modified dipping method described in Part I. The insects were separated from the suspension by means of a brass mesh, and were at once dried on the mesh by an air stream at 30° C. for $2\frac{1}{2}$ min., and kept in clean glass containers at 100% R.H. and either 25 or 12° C.

This avoids any effect humidity may have on toxicity (Pradhan, 1949). Inspection was made after 24 hr.; Fig. 1, graphs B and C, and Table 1 show the results, calculated for best fit. Again, needle aggregates are more toxic than colloidal DDT at 25° C. (3·8–7·9 times), but at 12° C. the ratio is reversed, the colloid being slightly more toxic (1·7–1·8 times). Each form has a negative temperature coefficient, but for colloid the coefficient is bigger.

Thus with both species, the relative potencies of DDT suspensions clearly depend on temperature after treatment. Quite naturally, the temperature responses of the two species are not arithmetically equal, but they are in the same direction. Colloidal DDT is less toxic than needle aggregates to warm insects, but it is the more toxic to cool insects.

TABLE 1. *Results of dipping tests, showing effect of temperature on relative toxicity of DDT colloid and needle aggregates to adult* Oryzaephilus surinamensis (*test A*) *and to adult* Tribolium castaneum (*tests B and C*).

Test	Suspension type	Mean size (μ)	Temperature of test (° C.)	Equation	Ratio of M.L.C. (colloid : needle agg.)
A	Colloid	—	12	$y=1{\cdot}71x+3{\cdot}30$	0·13
	Needle agg.	417	12	$y=2{\cdot}76x-0{\cdot}21$	
	Colloid	—	30	$y=2{\cdot}37x-1{\cdot}82$	5·4
	Needle agg.	417	30	$y=1{\cdot}92x+0{\cdot}88$	
B	Colloid	—	12	$y=4{\cdot}81x+0{\cdot}78$	0·60
	Needle agg.	435	12	$y=5{\cdot}63x-1{\cdot}18$	
	Colloid	—	25	$y=2{\cdot}35x-1{\cdot}18$	7·9
	Needle agg.	435	25	$y=3{\cdot}26x-0{\cdot}65$	
C	Colloid	—	12	$y=3{\cdot}35x+1{\cdot}76$	0·57
	Needle agg.	437	12	$y=4{\cdot}74x-0{\cdot}75$	
	Colloid	—	25	$y=1{\cdot}64x+1{\cdot}21$	3·8
	Needle agg.	437	25	$y=3{\cdot}78x-1{\cdot}51$	

TEST BY APPLICATION OF MEASURED DROPLETS OF SUSPENSIONS

Large milkweed bugs (*Oncopeltus fasciatus* Dall.) are rather resistant to DDT, and cannot be killed by application of measured droplets of DDT suspensions to the outside of the cuticle. On the other hand, rotenone kills them easily, and their reaction to different particle sizes can be measured in this way.

Method. Droplets of 0·002 ml. of rotenone suspensions were applied to CO_2-anaesthetized adult milkweed bugs, under the wings, with a 1 ml. syringe and blunt 27-gauge needle (Beard, 1949*a*). The suspension medium was 0·1% saponin in water, but to reduce sedimentation of crystals in the syringe, 0·125% of sodium alginate was also included in all suspensions, and crystalline suspensions were renewed in the syringe every seventh insect. After treatment, the insects were kept at 27° C., with access to water and dried milkweed seeds, and were inspected several times until the kills stopped increasing. Control deaths were few.

Results. Fig. 1, graph D, shows the results of a test with colloidal rotenone and small hexagons of rotenone (Part II). Probit kill is plotted against log (percentage concentration × 10^4). The lines in this and all following graphs were fitted by eye. Most of them represent from four to six points, but for simplicity the points have been left out in nearly all cases. The vertical marks crossing the lines show the highest and lowest concentrations used. The time in days between treatment and

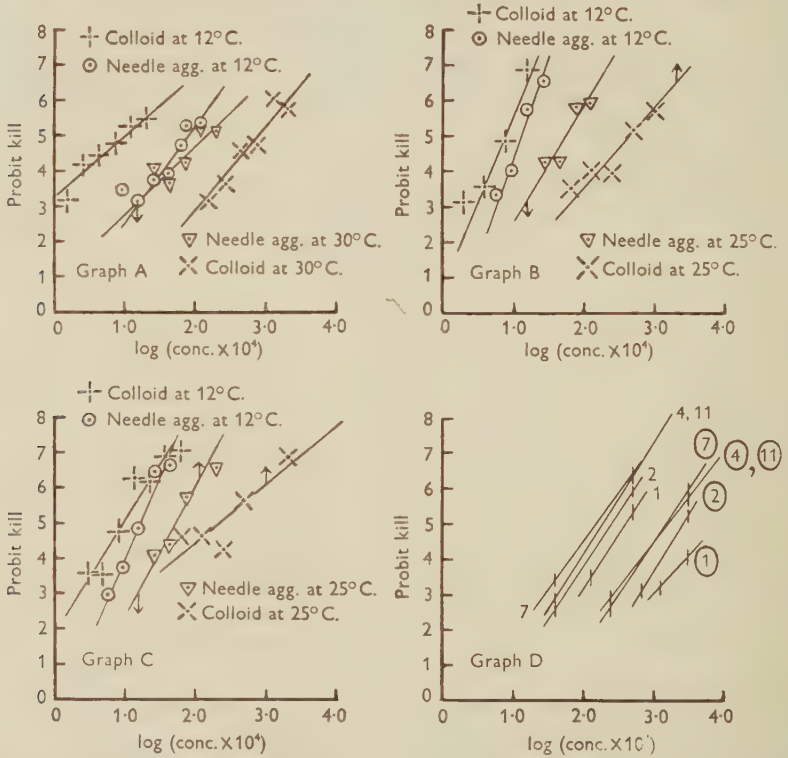

Fig. 1. Graph A. Mortality of adult *O. surinamensis* 1 day after dipping in DDT suspensions. Graphs B and C. Mortality of adult *T. castaneum* 1 day after dipping in DDT suspensions. Graph D. Mortality of adult *O. fasciatus* at various times after application of rotenone suspensions. The time intervals in days are shown at the ends of the lines, ringed for small hexagons, plain for colloid.

inspection is shown at the end of each line, ringed for crystals, plain for colloid. Values for log (M.L.C. × 10^4) were obtained by interpolation from the graphs, and are shown in Table 2, along with their ratios (small hexagons : colloid).

Colloidal rotenone is more toxic than small hexagons, and both types give kills which increase between inspections, eventually reaching 'end-points' (Beard, 1949*b*). The kill from the small hexagons increases more rapidly than the kill from

the colloid; the toxicity ratio decreases to a steady value. There is no recovery of the kind seen in Part II with colloidal rotenone. However, the general reaction, namely, higher kill from colloidal rotenone and decreasing toxicity ratio with the passage of time, is the same as that of *Oryzaephilus surinamensis* in Part II.

TABLE 2. *Results of application test D, showing values of log* (M.L.C. × 10^4) *for rotenone suspensions against adult milkweed bugs* (Oncopeltus fasciatus)

Time in days	log (M.L.C. × 10^4) for Colloid	log (M.L.C. × 10^4) for Small hexagons, 30 × 21 μ	Ratio of M.L.C. (small hex.: colloid)
1	2·55	3·90*	(22)
2	2·36	3·38	10
4	2·23	3·19	9·1
7	2·16	3·15	9·8
11	2·24	3·21	9·3

* By extrapolation.

EFFECTS OF TEMPERATURE ON TOXICITY OF SUSPENSIONS BY INJECTION

Some tests have been made by injecting suspensions of rotenone, DDT and the fluorine analogue of DDT, often called DFDT (Metcalf, 1948). These tests are now described. DFDT was included because of its high lipoid solubility. A detailed discussion of the theory of the injection method, with an explanation of the results, is given in Part IV.

Methods. Adult milkweed bugs under CO_2 anaesthesia were injected parenterally with 0·002 ml. suspension from a 1 ml. syringe with a 27-gauge needle. As before, sedimentation was avoided by renewing the crystalline suspensions every seventh insect, and by including 0·125% sodium alginate in all suspension media (0·1% saponin for rotenone, and 0·1% sulphonated lorol for DDT). Forty insects were used at each concentration; and they were kept at either 27 or 10° C. after treatment, with water and milkweed seeds.

The methods for making the suspensions are given in Part I (DDT) and Part II (rotenone). A colloidal suspension of DFDT can be made by the method described for colloidal DDT (Part I). The following method gives a crystalline suspension. 0·1 g. DFDT (m.p. 40–41° C.) is dissolved in 10 ml. 95% alcohol; 170 ml. water containing 0·2 g. sulphonated lorol (or, preferably, 'Dreft') is added with stirring to the alcohol at 2 drops per second until a milkiness appears, and then more quickly until addition is complete. The mixture, when stirred for 24 hr. at 5–10° C., precipitates needles of uniform size (60μ). Lastly, 20 ml. of water, containing 0·25 g. sodium alginate is added. The final composition is DFDT 0·05%, sulphonated lorol 0·1%, sodium alginate 0·125% and alcohol 5%.

Results. The results of the tests at 27° C. are given as probit lines, fitted by eye, in graphs E–H (Fig. 2). The rates of increase of kill at 10° C. were followed until the kills stopped increasing, and the results are given in graphs I–N (Figs. 3, 4),

with the time interval in days and the range of concentrations shown in the same way as in graph D (application test). The broken lines are fixed by only two points each. Corrections have been made where necessary for deaths in the controls, which seldom exceeded 20% even 2 weeks after treatment, and were usually less than 10%. Values for log (M.L.C. $\times 10^4$) were obtained by interpolation from the graphs, and are shown in Table 3, along with their ratios (crystalline suspension: colloid).

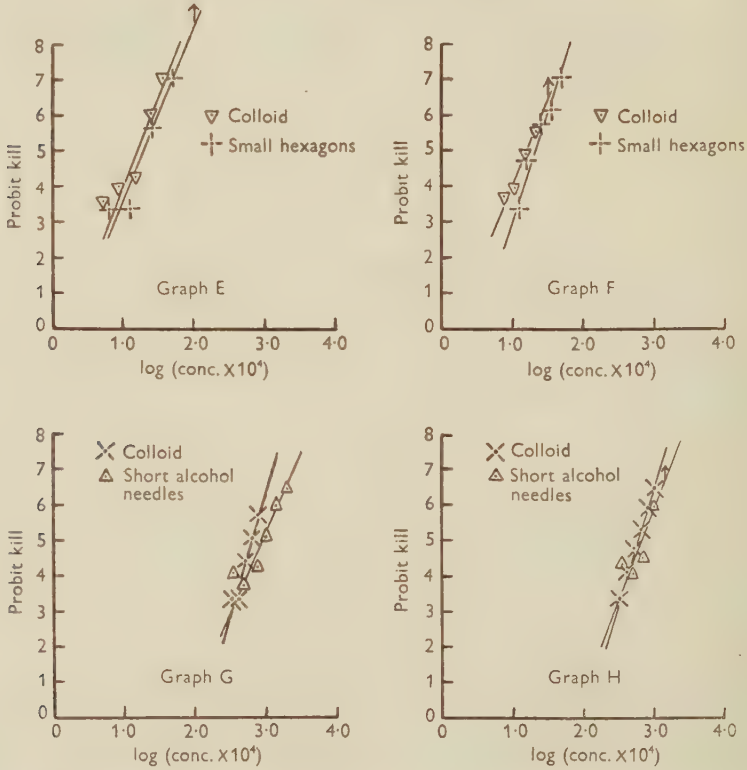

Fig. 2. Graphs E and F. Mortality of adult *O. fasciatus* 2 days after injection of rotenone suspensions (27° C.). Graphs G and H. Mortality of adult *O. fasciatus* 3 (graph G) and 2 (graph H) days after injection of DDT suspensions (27° C.).

When a suspension of rotenone small hexagons is compared with colloidal rotenone in this way, and the insects are kept at 27° C. after treatment, the two types are almost equitoxic after 2 days (Fig. 2, graphs E and F, and Table 3). The very slight difference in toxicity may simply be due to a small amount of sedimentation in the syringe.

In similar tests, a suspension of DDT short alcohol needles is equitoxic with colloidal DDT after 2 or 3 days at 27° C. (Fig. 2, graphs G and H, and Table 3).

When insects are injected with rotenone small hexagons or colloid and kept at 10° C. after treatment, the colloid seems to be very much more toxic than the crystalline suspension after 2 days (Fig. 3, graphs I and J, and Table 3). The ratio is not easy to measure, as the small hexagons give a very flat line. Afterwards, as

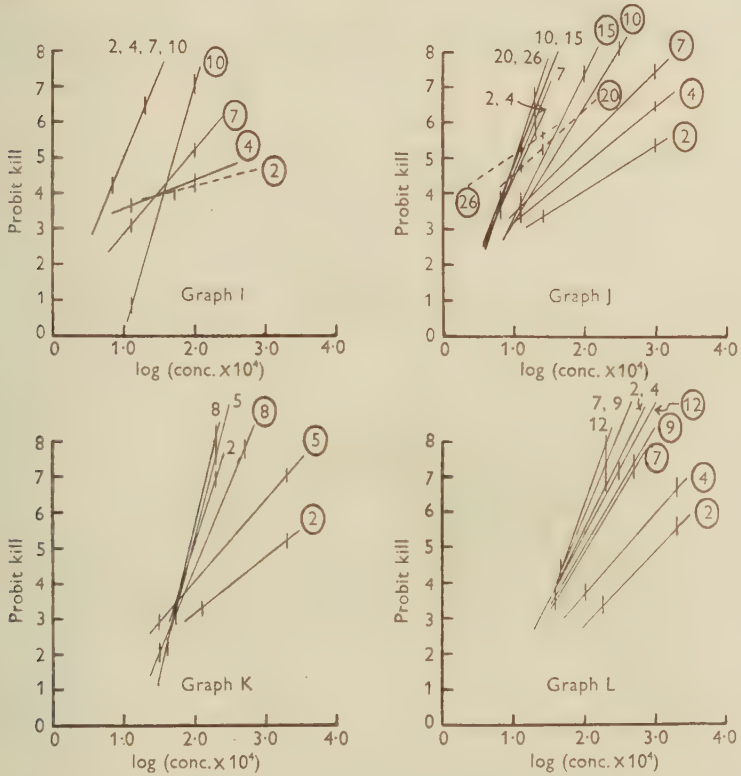

Fig. 3. Graphs I and J. Mortality of adult *O. fasciatus* at various times after injection of rotenone suspensions (10° C.). The time intervals in days are shown at the ends of the lines, ringed for small hexagons, plain for colloid. The broken lines are fixed by only two points each. Graphs K and L. Mortality of adult *O. fasciatus* at various times after injection of DDT suspensions (10° C.). The time intervals in days are shown at the ends of the lines, ringed for short alcohol needles, plain for colloid.

time passes, the kill from the colloid increases very slowly indeed, but the kill from crystalline rotenone increases throughout the test, quickly at first, and then more slowly. At the same time, the slope of the probit line increases. The ratio of M.L.C. is about 4 after 10 days, and after about 3 weeks the kills from the two types become roughly equal.

TABLE 3. *Results of injection tests E–N, showing effects of temperature and time on toxicity of suspensions to adult milkweed bugs* (Oncopeltus fasciatus)

Test	Temp. of test (° C.)	Poison	Crystal size (μ)	Time (days)	log (M.L.C. × 10^4) for Colloid	log (M.L.C. × 10^4) for Crystals	Ratio of M.L.C. (crystals : colloid)
E	27	Rotenone	37 × 23	2	1·20	1·28	1·2
F	27	Rotenone	39 × 29	2	1·20	1·32	1·3
G	27	DDT	67	3	2·80	2·95	1·4
	27	DDT	47	2	2·74	2·81	1·2
	10	Rotenone	41 × 30	2	1·00	3·60*†	(400)
				4	1·03	2·78†	(56)
				7	0·98	1·88	8·0
				10	0·97	1·70	5·4
	10	Rotenone	41 × 30	2	1·12	2·69	37
				4	1·10	2·08	9·6
				7	1·08	1·77†	4·9
				10	1·03	1·56	3·4
				15	1·07	1·40	2·1
				20	1·02	1·22*	(1·6)
				26	1·06	0·96*†	(0·8)
K	10	DDT	55	2	1·96	3·14	15
				5	1·94	2·40	2·9
				8	1·93	2·11	1·5
L	10	DDT	58	2	1·86	3·06	16
				4	1·88	2·56	4·8
				7	1·81	2·03	1·7
				9	1·81	1·99	1·5
				12	1·78	1·88	1·3
M	10	DFDT	59	2	2·80	2·73	0·85
				6	2·66	2·54	0·76
N	10	DFDT	60	2	2·48	2·61	1·4
				5	2·49	2·57	1·2
				7	2·47	2·55	1·2

* Line fixed by two points only. † By extrapolation.

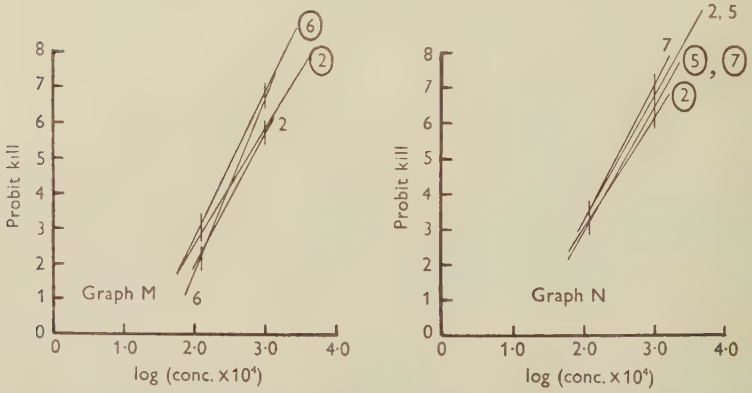

Fig. 4. Graphs M and N. Mortality of adult *O. fasciatus* at various times after injection of DFDT suspensions (10° C.). The time intervals in days are shown at the ends of the lines, ringed for needles, plain for colloid.

When colloidal DDT and short needles are compared, using milkweed bugs at 10° C., the colloid is more toxic than the crystals by about 15 times after 2 days. The kills become the same after about 10 days (Fig. 3, graphs K and L, and Table 3).

Colloidal DFDT and 60μ needles of DFDT are equally toxic in 2 days to milkweed bugs at 10° C. Afterwards, the kills from the two suspensions increase slowly, and at the same rate (Fig. 4, graphs M and N, and Table 3). The behaviour of DFDT in insects at 10° C. is, in fact, the same as that of rotenone or DDT at 27° C.

Discussion

Although an explanation and discussion of the results of this and earlier papers are given in Part IV of this series, it is convenient to make a few comments here.

(*a*) There is one clear difference between contact and injection tests. In tests of rotenone by dipping (Part II) and by application, the differences in toxicity between colloidal and crystalline suspensions persisted; they became a little smaller during the tests, but never disappeared. But in the injection tests, colloidal and crystalline poisons differed, if at all, only in their speeds of action. The final kill from each type was the same.

(*b*) In Part I, it was noted that in dipping tests DDT needle aggregates are retained more efficiently than colloidal DDT by *Tribolium castaneum*, and that the difference in efficiency of retention is nearly enough to make up for the difference in toxicity at 27° C. When the suspensions were compared on the basis of weight of poison retained, and not on concentration applied, they were of almost equal toxicity. It now seems (p. 568–9) that the conditions chosen in Part I were, by chance, rather fortunate. If the toxicity tests had been made at a lower temperature, then correction for retention, whether made by the method I used or by the refined method of Stringer (1948), might in fact have caused the probit lines to separate rather than approach or overlap. Stringer further suggested that a difference in slope of probit lines found in contact tests (Part I) could be explained by a difference in 'availability coefficients', which serve to express the amount of poison retained by insects from external treatment. The graphs of the contact time-mortality tests in Part II and of injection tests of crystalline suspensions in this paper show that in both types of test the probit lines increased in slope as time passed. The same increase in slope with time has been found by Beard (1949*b*) in tests on milkweed bugs by topical application of nicotine and by injection of parathion. In application and injection tests there can be no question of differences in retention. So, in tests of different forms of one poison against one species by one method at one temperature, *differences* in slope may easily be connected with differences in speed of action and be quite independent of retention. It may not always be possible, in fact, to name the cause with certainty.

(*c*) The results of this paper show that lowering the temperature of a test usually

increased the relative toxicity of small particles. Häfliger (1949) found that when DDT crystals of different sizes were fed in suspension to honey-bees, the smallest crystals were always the most toxic, the relative toxicities being independent of temperature in the range 20–38° C. If the bees had been kept colder still, perhaps the difference in relative potency might have increased.

I would like to thank Dr Friend and his colleagues in New Haven, and Dr Tattersfield and Dr Potter at Rothamsted, for their helpful suggestions.

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THE INFLUENCE OF HEAT ON SOME APHIDS

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(With 2 Text-figures)

The thermal death-points of five species of aphids removed from their host plants lay between 38 and 41° C., when tested for 1 hr. at 60% R.H. Many aphids alive after 1 hr. at high temperatures died within the next day; no *Myzus persicae* recovered and reproduced after 1 hr. above 37·5° C. Third and fourth instars and adult apterae withstood heat better than first and second instars and alatae. More aphids died at 90% R.H. than at 60% R.H., and more at 60% than at 30% R.H. Aphids kept at 15% R.H. for 4 hr. before being heated showed a higher mortality than those kept at 95% R.H. Aphids on plants withstood temperatures higher than their thermal death-point off the plant. Presumably aphids can continue to cool themselves by evaporation while feeding; also lower temperatures on the surface of transpiring plant tissues will aid survival.

Heat affects aphids in many ways, influencing movement, rate of development, rate of reproduction, number of nymphs born per adult, and length of life. It is accepted that aphids do not flourish at high temperatures; Fenjves (1945) found that temperatures over 28° C. were detrimental for *Myzus persicae* (Sulz.), but there is little information on their thermal death-points. Chaine (1919) noticed that a sudden rise in temperature to 37° C., or above, caused whole populations of *Macrosiphum rosae* (L.) on roses to die rapidly, whereas a slower rise to the same temperature produced much less effect. Spencer (1926) watched the reactions of *Myzus persicae* on potato plants at different temperatures, and found that general movement began at 33° C., and that the aphids dropped from the plants at 40° C.

The effects of heat on five species of aphids have been determined, using apparatus similar to that described by Mellanby (1932). The insects were confined in a copper gauze cage, fixed to the end of a thermometer, and lowered into a bottle containing a small amount of an appropriate constant humidity mixture. Saturated solutions of sodium monoacid phosphate (giving 90–93% R.H.), sodium bromide (59–62% R.H.), and calcium chloride (30–35% R.H.) were used for this purpose. The testing bottle was immersed in a large water-bath, thermostatically controlled. Fresh aphids, reared in a glasshouse and taken directly from the plants, were used for each test. The aphids were kept for 1 hr. at the test temperature, then examined in a small Petri dish under a low-power binocular microscope. All aphids showing movement were recorded as alive, even though some were obviously moribund, and others did not survive a further 24 hr. Thus, although all *Brevicoryne brassicae* (L.) were alive at the end of 1 hr. at all temperatures up to and including 40·5° C., some died within 24 hr. after tests at 38·5–39·5° C., while none survived the 24 hr. after tests at 40·0

* Now at Ministry of Agriculture and Fisheries.

and 40·5° C. Tests for survival were made in closed Petri dishes at 15° C. and about 70% R.H. In the five species tested, the temperatures at which all survived the test differed by 1–3° C. from the temperatures at which all aphids died. The thermal death-points have been taken as the temperatures at which some aphids died within the test hour at 60% R.H.; for the species examined these temperatures were:

Aulacorthum solani (Kalt.)	38·0° C.
Acyrthosiphon pisum (Harris)	38·0° C.
Macrosiphum euphorbiae (Thomas)	38·5° C.
Myzus persicae (Sulzer)	38·5° C.
Brevicoryne brassicae (L.)	41·0° C.

TABLE 1. *Aphids alive after 1 hr. at various temperatures at* 60% R.H.

	B. brassicae		*M. persicae*		*M. euphorbiae*		*A. pisum*		*A. solani*	
Temp. (°C.)	No. tested	% alive	No. tested	% alive	No. tested	% alive	No. tested	% alive	No. tested	% alive
36·0	7	100	8	100	10	100	10	100	24	100
36·5	5	100	6	100	8	100	6	100	29	100
37·0	9	100	28	100	13	100	11	100	30	100
37·5	15	100	50	100	20	100	20	100	25	100
38·0	67	100	63	100	26	100	24	96	19	63
38·5	65	100	124	98	37	92	11	82	60	15
39·0	87	100	102	74	35	26	38	55	33	12
39·5	24	100	109	39	35	3	13	38	31	0
40·0	20	100	34	6	56	0	13	0	—	—
40·5	14	100	37	3	—	—	—	—	—	—
41·0	60	58	42	0	—	—	—	—	—	—
41·5	26	27	—	—	—	—	—	—	—	—
42·0	27	0	—	—	—	—	—	—	—	—

TABLE 2. *The survival for 24 hr. of aphids which withstood various temperatures for 1 hr. at* 60% R.H.

	B. brassicae		*M. persicae*	
Temp. (°C.)	No. tested	% alive	No. tested	% alive
37·0	—	—	19	100
37·5	—	—	29	90
38·0	43	100	35	86
38·5	45	76	81	72
39·0	57	68	48	38
39·5	24	46	12	0
40·0	10	0	—	—
40·5	14	0	—	—
41·0	8	0	—	—

Table 1 shows the number of aphids alive after tests of 1 hr. at different temperatures and 60% R.H. Table 2 records the survival for 24 hr. of *B. brassicae* and *Myzus persicae* which withstood these tests.

Although at 38° C. and below, all the *M. persicae* survived the test hour, and most survived for a further 24 hr., survival after these tests was no guarantee of ability

to recover if returned to the host plant. When *M. persicae* from turnips were tested for 1 hr. at 37·5, 38·0 and 38·5° C., and those alive at the end of the test were then placed in small cages on the underside of turnip leaves, their recovery was largely dependent on the temperature of testing (see Table 3). It will be seen that after exposure to 38·0 and 38·5° C. none of the aphids reproduced, and almost all died within 72 hr. Only those aphids which appeared unaffected by the heating were tested for their ability to feed and reproduce on the host plant. After each test there were usually some aphids showing unco-ordinated movement; these were found to die within 36 hr.

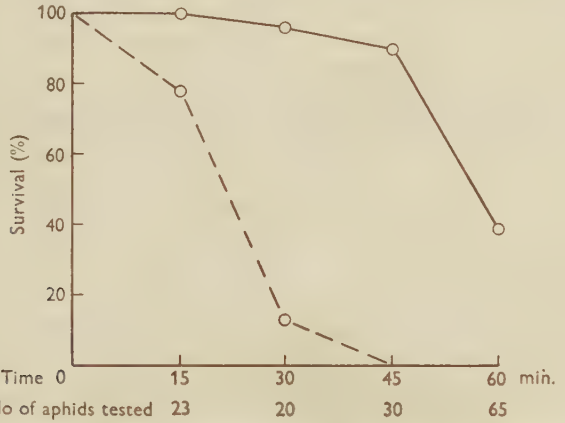


Fig. 1. *M. persicae* alive after various times at 39·5° C. and 60% R.H. ○——○, alive at end of test (%); ○——○, alive 24 hr. after test (%).

All instars were used in the tests, including nymphs of alatae, but not adult alatae. Differences between instars were not great, but from a total of all tests in which some aphids had died and some had survived, it was evident that a greater proportion of first and second instars had succumbed than third, fourth and fifth instars (Table 4). Adult apterae withstood heat better than adult alatae: in comparative tests with *M. persicae*, 75% of the apterae (30/40) were alive after 1 hr. at 60% R.H., compared with 33% of the alatae (16/49). In a test with *Brevicoryne brassicae*, 83% of the apterae (21/26) were alive after 1 hr. at 41·0° C. and 60% R.H., compared with 26% of the alatae (8/31). To find at what time during the test hour exposure to temperature killed, batches of *Myzus persicae* were subjected to 39·5° C. and 60% R.H. for periods of 15, 30, 45 and 60 min. Most aphids were alive after 45 min. but not after 60 min. (Fig. 1). The effect was thus greatest during the last 15 min. of the hour, though most of the survivors from 30 and 45 min. heating died within the next 24 hr.

The effect of heat on *M. persicae* was tested at different humidities (Fig. 2). More aphids were alive after 1 hr. at low humidity (30% R.H.) than at high (90% R.H.), and the higher the temperature the greater the protecting effect of low

humidity. This suggests that survival of heat depends largely on the water balance within the insect; it will survive higher temperatures, or longer at the same temperature, when it can cool itself by evaporation than when the humidity is high

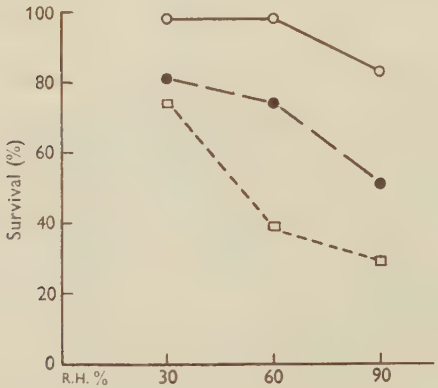
TABLE 3. *Recovery of* Myzus persicae *after heat treatment*

Temp. (R.H. = 60 %) (° C.)	Hours after return to host plant following 1 hr. heat treatment: 24 Dead	24 Alive	48 Dead	48 Alive	72 Dead	72 Alive
37·5	1	15 (R)	2	14 (R)	7	9 (R)
38·0	9	71	53	27	79	1
38·5	0	21	21	0	21	0

(R) = producing nymphs.

TABLE 4. *The survival of different instars of all the species used in all the tests in which some were alive and some were dead after exposure to critical temperatures for 1 hr.*

Instar	Total tested	Percentage alive
1 + 2	228	39
3	235	59
4	270	55
Apterae 5	234	56
Alatae 5	80	30



No. of aphids tested	38·5° C.	39·0° C.	39·5° C.
R.H. % 30	128	55	57
R.H. % 60	124	102	109
R.H. % 90	95	143	180

Fig. 2. *M. persicae* alive after 1 hr. at various temperatures and humidities.
○——○ 38·5 C. ●— —● 39·0 C. □- - -□ 39·5 C.

and evaporation is reduced. When the aphids were pre-treated by being kept at either 15 or 95 % R.H. at 15° C. for 4 hr. before being tested at 39·0° C. and 60 % R.H., more of those which had been pre-treated at 15 % R.H. died than of those

which had been pre-treated at 95% R.H. (Table 5). It seems likely that pre-treatment at low humidity reduced the amount of water available within the insects for cooling themselves by evaporation during the test.

When aphids were allowed to remain on the plants and replenish their water, their thermal death-points were higher than those recorded above. *M. persicae* and *Brevicoryne brassicae*, feeding on cauliflower, were placed in a glass chamber, the temperature of which could be controlled. During 1 hr. at 32° C. there was no movement. The temperature was then raised to 38° C. over a period of 15 min.; at 34–35° C. some of the aphids became restless; at 35–36° C. some began to drop from the plants. In two tests, 64 and 66% of the *B. brassicae*, and 61 and 32% of the *Myzus persicae*, remained on the plants and survived 1 hr. at 37–38° C.

TABLE 5. Myzus persicae *tested for* 1 *hr. at* 39·0° C. *and* 60% R.H. *after* 4 *hr. pre-treatment at* 15° C. *and* 15 *or* 95% R.H.

	15% R.H.	95% R.H.
No. of aphids tested	49	51
Percentage alive after test	16	61

During 30 min. at 42–43° C. most of the aphids fell off and died, but those which remained on the plants survived and were alive and reproducing 2 days later. Presumably aphids can cool themselves by evaporation when feeding; also the temperatures on the surfaces of transpiring leaves are lower than that of the air, and these lower temperatures will aid survival. Aphids more readily left the leaves which wilted than leaves which did not. This might explain the observation of van der Plank (1944) that *M. persicae* left potato plants, which wilt easily, when the mean daily maximum reached 32° C., whereas they continued to infest cabbage plants in the same field. Ambient air temperatures in Britain rarely reach the thermal death-point, but exposure of the aphids to radiant heat might raise the body temperature of the insects by 5–15° C. (Gunn, 1942) above the air temperature, and might cause death. This may be one factor causing many aphids to occur predominantly on the lower surfaces of leaves, where they are not directly exposed to sunshine.

Mr I. Long kindly ascertained the relative humidities within the test vessels.

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AN IMPROVED SUCTION TRAP FOR INSECTS

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(With Plate 14 and 2 Text-figures)

A suction trap for airborne insects, which automatically segregates the catch at pre-set intervals and standardizes the samples for volume of air, has been previously described by Johnson. Experience with this trap has shown that certain structural improvements were advisable. These have now been incorporated. Constructional details are given and the design of larger traps discussed. It is shown that aphids and lacewings taken are in good condition.

Introduction

When sampling populations of flying insects it is desirable to use a trapping method as independent as possible of wind-speed and other meteorological factors. It is also essential to the study of flight periodicity to know the time of arrival in the trap of any particular insect. Moreover, these facts should be obtained automatically if sampling on a large scale or over long periods is contemplated. The trap should be easily cleaned and reset. The catch should also be clean and easily sorted.

Johnson (1950*a*, *b*) described a suction trap in which these features are prominent. The insects are drawn down a gauze cone into a collecting tube by a ventilating fan mounted with the blades in a horizontal plane. Disks, coated with a lethal film of pyrethrum, are dropped at regular intervals into the collecting tube, segregating the catch. The time-period of the disk-dropping mechanism is decided by a time-switch, the entire operation being automatic and electrically controlled. The time-switch can be chosen to drop disks at almost any interval, although space limitations decide that not more than about thirty disks can be loaded at a time. In practice twenty-five disks have been used, allowing 1 day's trapping with hourly periods, or 3 days' trapping with 3-hourly periods.

The prototype described by Johnson worked perfectly under laboratory conditions and has produced excellent results during two seasons in the field. It has, however, some inherent weaknesses owing to the light construction of the disk-release mechanism, and these have been accentuated by long exposure to bad weather conditions. A more robust instrument has therefore been designed which also gives greater protection from the weather and from possible damage in handling.

Constructional details

There were two main weaknesses in the original model:

(1) The 'gate', which supports the disks, was pivoted singly (see Johnson, 1950*a*, Text-fig. 2, *K*), and the torque produced was aggravated by friction between jaws and disks, and between disks and column. These factors, in turn, were accentuated by damp and dust until a stage was reached when disks would no longer fall. It was then necessary to reduce the solenoid gap to obtain more power, and then to increase the spring tension to equalize it. Since it was impossible to do this without affecting the position of the jaws relative to the disks it became increasingly difficult to obtain consecutive samples without constant attention.

(2) When the trap was unloaded by an inexperienced operator there was a certain danger of the catch being upset and also of the collecting tube being distorted so that the disks would not fit perfectly when next loaded.

The improvements found necessary may be listed as follows:

(1) The 'gate' was previously suspended from a single pivot which was situated out of line with the point of contact between jaws and disks. The gate has now been pivoted bilaterally removing the torque produced by this single suspension.

(2) The disk-release mechanism was formerly operated by a 15 V. hand-wound solenoid in a transformer circuit. A mains-operated solenoid is now used, making the transformer obsolete, giving more latitude to mechanical adjustment and more power to overcome friction between the jaws and the disks.

(3) Independent adjustment has been given to the left and right action of the jaws, making it possible to change the position of the jaws relative to the disk they are holding without affecting the action of the solenoid and return spring.

(4) The column down which the disks slide is now rustless, removing the danger of disks lodging when long periods of wet weather are encountered.

(5) The solenoid and return spring have been housed above the column where they are enclosed and protected.

(6) The dropping mechanism and loaded disks have been protected from dust, insects, rain, etc., by a cellophane shield.

(7) The centring plate, which was originally a flat disk clamped against the end of the collecting tube (see Johnson, 1950*a*, Text-fig. 1, *J*), is now made into a cap which fits over the end of the collecting tube thus preventing lateral displacement when removing the catch.

(8) The collecting tube, formerly of gauze (Johnson 1950*a*, Text-fig. 1, *C*), is now of brass tubing with gauze panels and consequently much less fragile. It is shielded by an outer tube, also perforated, for further protection.

Detailed description

The parts are made, as far as possible, from stock sizes of brass rod, tube and strip. Standards of turning and fitting required are not high, but reasonable accuracy

must be maintained in aligning the jaws with the column and the column with the collecting tube. Measurements are to fit 9 or 12 in. Vent-Axia fans unless otherwise stated.

For convenience the trap is described in three sections:

(1) The disk-release mechanism, including all the internal details.
(2) The air filtering cone; i.e. the cone itself and the fixed cylinder at its base.
(3) The removable collecting tube and cap.

(1) *The disk-release mechanism* (Pl. 14, figs. 1, 2, and Text-fig. 1)

This comprises the solenoid housing, the column, the pivots and the gate.

The solenoid housing itself is built up from two disks or base plates (*M* and *O*) of $\frac{1}{8}$ in. duralumin plate spaced apart by four support pillars (*N*) each 2 in. long, cut from $\frac{7}{16}$ in. brass rod, drilled through and tapped. The diameters of the plates are arbitrary, depending on the shape of the fan casing, but the lower one is less than the upper and both are tapered to allow the shield (*Z*) to be in the form of a truncated cone.

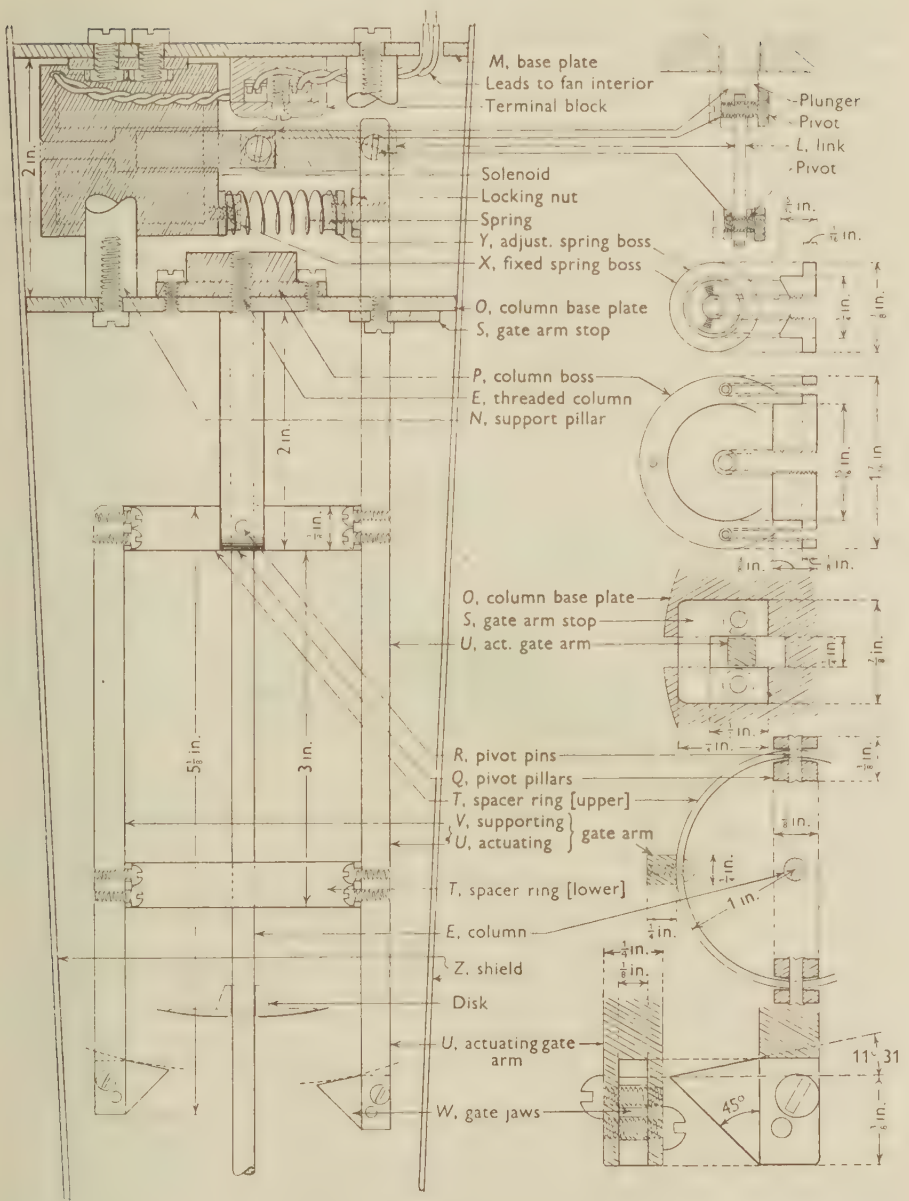
To the base plate (*M*) is bolted the solenoid, mounted horizontally, and the terminal block. The plate *M* is bolted direct into the fan casing. Details will depend upon the fan in use. The leads from the solenoid are taken into the terminal block and from there through the plate *M* and the fan casing, both drilled for this purpose. Inside the fan they are connected, along with the fan motor leads, to a four-core cable introduced through the lead-duct built into the fan.

The column base plate (*O*) is slotted to allow movement of the actuating gate arm (*U*), and to it are bolted the pivot pillars (*Q*), the gate arm stop (*S*) and the column boss (*P*). It is also drilled and tapped to receive the column. The column (*E*) is a length of $\frac{3}{16}$ in. diam. Monel rod threaded at each end. The column boss (*P*) is turned from $1\frac{1}{2}$ in. diam. brass rod and is drilled centrally and tapped to take the column. It is also drilled and tapped at four points around the flange to take 4 B.A. bolts. The column is first screwed into the plate *O* and the boss *P* screwed down to lock the column. The plate is then drilled through the bolt holes in the boss flange and cleared to allow insertion of four bolts from below or tapped to receive bolts screwed in through the boss from above. The column is thus locked into position vertical to the plate, its weight being taken by the boss.

The pivot is formed by the two pivot pillars (*Q*) made from 2 in. lengths of $\frac{3}{8}$ in. square brass bar, which are bolted to the plate *O*. They are slotted to receive the upper spacer ring (*T*) and drilled to allow insertion of short $\frac{1}{8}$ in. diam. steel pins (*R*). These are the bearings. The ring *T* is also drilled to receive these pins and pivots upon them. After final adjustment the pins are cut off and split.

The gate is a rigid structure built from two arms of $\frac{1}{4}$ in. square-section brass, held apart by two $\frac{1}{16}$ in. thick brass rings. It serves to transmit the action of the solenoid to the jaws (*W*).

The supporting gate arm (*V*) and the actuating gate arm (*U*) are $5\frac{1}{4}$ and $8\frac{1}{2}$ in.



Text-fig. 1. Partial section of disk-release mechanism. Details to same scale as sectional drawing except for the spring boss and gate jaws which are × 2.

lengths of $\frac{1}{4}$ in. square brass respectively. The arm V is bolted to the spacer rings and slotted at its lower extremity to receive the jaw. The arm U projects somewhat farther at the bottom than V and is continued upwards, through the plate O to a point opposite the solenoid plunger. Here it is slotted and drilled to make a pivoted connexion with the solenoid plunger link (L). It is also drilled and tapped to take the adjustable spring boss (Y). The spacer rings (T) are $\frac{3}{8}$ in. lengths cut from brass tubing 2 in. outside diam. × 15 i.s.w.g. The jaws (W) are cut from $\frac{1}{8}$ in. thick brass strip and their shape is best seen from the diagram. They are inserted in slots in the gate arms and each is bolted twice. The under surfaces are steeply sloping to allow the disks to be pushed past when loading the trap. The upper surfaces are tangential to an arc centred where the gate is pivoted, so that disks are not jammed by the undue lifting or lowering of the point of contact when the jaws are in action.

The solenoid, as supplied, is not spring-loaded, hence a return spring must be mounted. This spring must exert a pressure equal to half that of the solenoid. The spring was made to a specification worked out from the rated power of the solenoid in use; but since the exact distance of the solenoid plunger from the gate arm may vary according to construction it seemed advisable to allow for adjustment here. The spring is therefore mounted between two bosses (X and Y), one of which is movable. The boss X is bolted into the solenoid casing. The boss Y is mounted on the end of a long bolt on to which is threaded a lock-nut. The bolt is screwed into the gate arm, threaded to receive it, and lateral movement is thus possible. The pressure exerted in each direction can be measured at the jaws by a spring balance and, when these are equal, the lock-nut tightened up and the projecting bolt cut off. Should loss of power in the solenoid be experienced at a later date, adjustment can easily be made.

The gate arm stop (S) is a 1 in. length of $\frac{3}{4} \times \frac{3}{32}$ in. brass strip, slotted in one side to receive the gate arm and drilled in the wings to take 4 B.A. bolts. The solenoid plunger link (L) is a length of $\frac{3}{8} \times \frac{3}{32}$ in. brass strip. One end is rounded, drilled and bolted into the slot in the solenoid plunger. The other end is left resting in the slot at the upper end of the arm (U) until final adjustments are made when it is bolted in place. It should pivot freely at both ends.

The shield (Z) is a truncated cone of cellulose acetate held open by the plates M and O. The plates are turned down so that the cone tapers from its seating at the base of the fan casing to an aperture of $2\frac{1}{2}$ in. at a point 3 in. below the jaws. It hangs from two brass lugs above the plate M, and is supported by an aluminium ring bolted to them.

To assemble the mechanism. Mount the pillars N, and the lugs, on the plate M. Fix the terminal block and the solenoid, along with its boss X and link L, into place. Bolt plate M to the fan casing. Bring out the leads from the fan interior to the block and connect up with the solenoid leads. Assemble the gate, the jaws W being left free in the sockets. Build up the plate O with column E, boss P and pivots Q. Mount the gate in the pivots leaving the pins loose. Adjust the vertical position

of the jaws in relation to each other so that a disk drops easily from one to the other and out. Drill and bolt the jaws into place. Insert the spring and bolt plate *O* to the pillars *N*. Adjust the spring tension. Holding the jaws, each in turn, to project $\frac{1}{4}$ in. under the edges of the disks, mark the position of the plunger link and gate arm stop. Mount these in position and split the pivot pins.

Lateral movement of the jaws is dependent on the length of the link *L* and the stop *S*. On a larger trap (unpub.) they were made adjustable, but this seems to be unnecessary since replacement, should it be required, is easily effected.

(2) The air filtering cone (Pl. 14, figs. 3, 4, and Text-fig. 2)

(Measurements to fit 12 in. Vent-Axia fan.) The gauze cone *F* is supported by a framework of struts and rings (Pl. 13, fig. 4) and terminates in a perforated brass cylinder into which is inserted the collecting tube. It is a truncated cone of 26-mesh copper gauze, $27\frac{1}{2}$ in. in vertical height with a diameter of $12\frac{1}{4}$ in. at its base and 2 in. at its apex. Sufficient overlap must be left at each end to allow for soldering to its retaining rings.

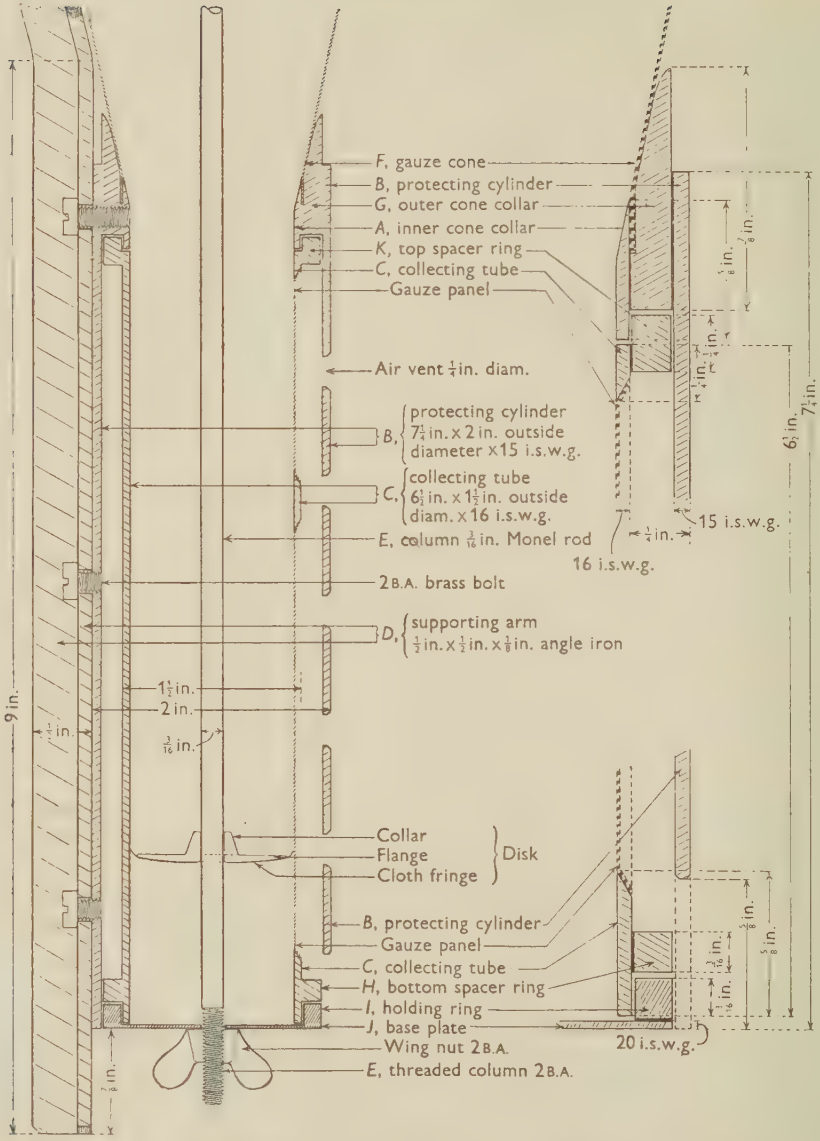
The supporting ring is of $\frac{3}{4} \times \frac{1}{8}$ in. brass strip. It is bent into a circle of 12 in. internal diameter, and in the case of the 12 in. Vent-Axia fan was bolted to the fan lugs which were exposed by cutting back the bakelite casing $\frac{1}{2}$ in. The cone is soldered to the ring and the joint banded by a retaining ring of $\frac{3}{4} \times \frac{1}{16}$ in. brass strip.

The three supporting arms (*D*) are made from $\frac{1}{8}$ in. thick $\frac{1}{2} \times \frac{1}{2}$ in. angle iron bent twice through $11\frac{1}{2}^\circ$ to give a sloping centre section of $25\frac{1}{2}$ in. with parallel end-pieces of 9 and $3\frac{3}{4}$ in. The $3\frac{3}{4}$ in. ends are bolted equidistantly around the retaining ring, the supports *D* converging towards the apex of the cone. Here the 9 in. sections are connected to the protecting cylinder (*B*). (From here onwards measurements are again suitable for 9 or 12 in. fans.)

This cylinder *B* is a $7\frac{1}{4}$ in. length of 2 in. outside diam. × 15 i.s.w.g. brass tubing perforated by six rows of $\frac{1}{4}$ in. diam. drill-holes allowing air to escape from beneath each dropping disk. Three pieces, each 1 in. × $\frac{3}{8}$ in., are cut from its lower end to allow removal of the collecting tube (*C*) (see Pl. 13, fig. 3). The cylinder *B* is bolted three times to each supporting arm, the uppermost bolt penetrating the outer and inner cone collars (*G* and *A*), between which is gripped the apex of the gauze cone. The collar *A* is a $\frac{5}{8}$ in. length of $1\frac{1}{2}$ in. outside diam. × 16 i.s.w.g. brass tubing chamfered inside its upper end to form a smooth outlet from the gauze cone. It projects $\frac{1}{8}$ in. below the collar *G*, which is turned from $1\frac{7}{8}$ in. outside diam. × $1\frac{1}{2}$ in. inside diam. brass tubing to fit inside the cylinder *B*. The basal quarter inch is a push fit on to the inner collar, but above it is turned out slightly larger to allow for the gauze.

(3) *The collecting tube and cap* (Pl. 14, fig. 3, and Text-fig. 2)

A piece of $1\frac{1}{2}$ in. outside diam. × 16 i.s.w.g. brass tubing $6\frac{1}{2}$ in. long (*C*) has six gauze panels let into it. These panels are of different lengths to allow escape of air at all levels and are $\frac{1}{2}$ in. wide. The edges of the panels are chamfered on the outside, the gauze being soldered and shaped to give a smooth interior of constant diameter.



Text-fig. 2. Partial section of base of cone with collecting tube inserted. Details are twice the scale of the sectional drawing.

At both ends of the tube *C* are mounted spacer rings (*H* and *K*), $\frac{3}{16}$ and $\frac{1}{4}$ in. deep respectively, turned from the same material as the outer cone collar *G*. They are a push fit *on to* the tube *C* to which they are soldered and a sliding fit *into* the

protecting cylinder *B*. The bottom ring (*H*) is inset a $\frac{1}{4}$ in. from the end of the tube and the top ring (*K*) projects $\frac{1}{8}$ in. The projecting lip thus formed at the top fits into the socket between the inner cone collar *A* and the protecting cylinder, whilst the projection at the bottom serves to centre the collecting tube cap.

This cap (called the centring disk, in Johnson, 1950*a*) is made from a base plate (*J*) of 20 i.s.w.g. brass soldered or bolted to a holding ring (*I*) identical with the bottom ring *H* of the collecting tube. When fitted to the tube it retains the disks and catch during loading and unloading operations. The cap is drilled centrally to allow the threaded column *E* to project, and a wing-nut holds the cap and collecting tube into the socket formed by the protecting cylinder.

Discussion

A trap of this design has been in constant use for 4 months, with only one breakdown which was caused by rotation of the solenoid face plate. As mentioned in the Appendix, this is easily remedied and applies only to the particular solenoid used.

Disks can be made by riveting or soldering a collar in the centre of a brass plate, but turning from solid brass is more satisfactory. In either case, the flange should be thin at the edges and the collar tapered towards the top (see Text-figs. 1 and 2).

The most satisfactory adhesive for the cotton disk-fringes in use to date is shellac cement which is soluble in alcohol. The lethal agent is therefore applied in petrol ether, and the insects removed by brush or by flotation in petrol ether or water.

The disks used are $1\frac{1}{4}$ in. in diameter and are held apart by a $\frac{1}{4}$ in. collar. This gives adequate spacing for the insects contained in samples of 20,000 cu.ft. of air in very high densities. One thousand aphids, along with other insects, have been readily accommodated on one of these disks.

Design of larger traps

If samples greater than 1000 aphids per period are required, the necessary adjustments to the disks and consequently to the dropping mechanism require considerable care. Powerful traps, using disks 3 in. in diameter, have been made, but it is better to increase the depth of the collar, rather than the disk diameter, where possible. Disks must be free to slide down the column, but with disks of diameter large in proportion to the depth of the collar the necessary play will produce disproportionate lift at the edge and this is the limiting factor in the alignment of the jaws. Disks 2 in. in diameter and $\frac{1}{2}$ in. deep should be ideal and would hold upwards of 5000 aphids. They should weigh about 40–50 g.

In places where a low density of insects is encountered it may be necessary to increase the volume of air sampled in order to obtain a statistically reliable catch. This may be done by any one, or a combination of all, of the three following means, each of which has its own advantages and drawbacks:

(1) Increase in the number of traps used, involving a considerable rise in initial outlay and slightly more time in emptying; but more desirable statistically.

(2) Increase in the speed of the fan. This is desirable in exposed positions and at high altitudes where strong cross-winds are encountered, since the fan speed should be at least equal to normal cross-wind speeds where possible. It has the disadvantage that with increased fan speeds an increase in damage to the catch may be expected. If very high fan speeds are essential it might be worth while having the collecting tube and disk-dropping apparatus above, i.e. upwind of the fan blades, but the difficulties in construction are obvious.

(3) Increase in the fan diameter. This would seem to be the most satisfactory solution for, although size itself may be a drawback, it is as well to remember that to increase the air sampled by five volumes it is necessary to increase the number of fans by five, the wind-speed by five, but the diameter by only just over two. Here again there is an increase in the peripheral speed of the fan blades, but this is slight compared with the faster fans and damage to the catch can be ruled out.

Damage to aphids in a 9 in. Vent-Axia fan is negligible, as it probably is to most other groups, with the possible exception of those prone to desiccation such as Cecidomyids, and a few of the larger insects which may occasionally be broken up.

Results obtained with two 9 in. segregating suction traps, one sited in a sheltered garden and the other in an open allotment, and both with fan speeds of 1250 r.p.m., show that in the groups so far dealt with this factor has presented no difficulties.

	Trap A	Trap B
Total aphids identified	28,687	6,766
Aphis fabae complex	25,432	4,572
Unidentifiable aphids	127	18
Haemerobiids identified and sexed	14	9
Chrysopids identified and sexed	2	11
Unidentifiable lacewings	0	0
Unsexable lacewings	1	0

Using fans revolving at 1200 r.p.m. in a rotating trap Williams & Milne (1935) were able to identify all groups encountered so that although the methods described have been developed especially for aphids they should be equally applicable to the majority of insects.

APPENDIX

Specifications for commercially produced items:

Solenoid: Westool a.c. solenoid, 2046 1, horizontal mounting, impulse rating, $\frac{5}{16}$ in. stroke, 1 lb. pull. (Obtained from J. H. Davies, 7 Vernon Drive, Stanmore, Middx.) The face plate of this solenoid is normally free to rotate around the plunger. It is therefore necessary to peg or cramp it tight before use.

Spring: width $\frac{3}{8}$ in., normal length $1\frac{1}{2}$ in., compressed length 1 in., resistance $\frac{1}{2}$ lb. between 1 and $1\frac{1}{4}$ in. (Obtained from Pocklington and Johnson, 72–74 Bridge Road East, Welwyn Garden City, Herts.)

Terminal block: Ex R.A.F. equipment Ref. No. 5C/430.

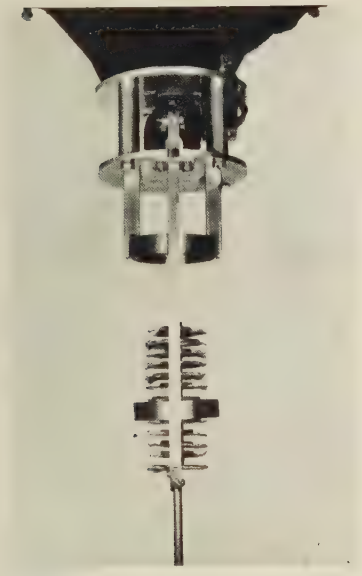


Fig. 1

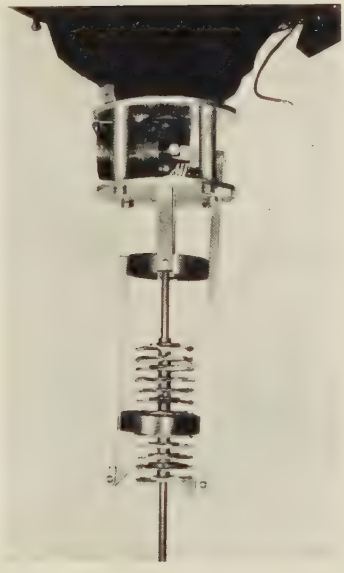


Fig. 2

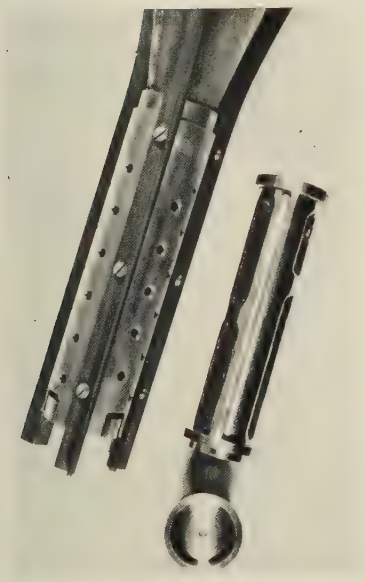


Fig. 3

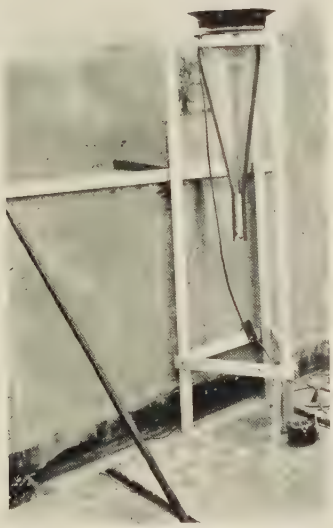


Fig. 4

I am grateful to Mr A. J. Arnold for instruction in lathe technique and to Dr C. G. Johnson for his help and encouragement.

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EXPLANATION OF PLATE 14

Figs. 1 and 2. Front and side view of disk-release mechanism with shield removed. Shield lugs are visible above the base plate. Prototype disks loaded.

Fig. 3. Base of cone with collecting tube and cap inserted; and spare tube and cap after removal.

Fig. 4. Trap using 12 in. Vent-Axia fan with 4-core lead to time-switch and mains.

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A STUDY OF THE BIOLOGY AND CONTROL OF *ANISOPUS FENESTRALIS* (SCOPOLI, 1763), A FLY ASSOCIATED WITH SEWAGE FILTERS

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(With Plate 15 and 6 Text-figures)

In work on the control of the sewage fly *Anisopus fenestralis* (Scopoli, 1763), it was shown that the larval phase was considerably lengthened by limiting the food supplied. The effects of food supply and temperature are considered in relation to the incidence of the adult fly in the filters. The assessment of the fly population of sewage filters by tray traps, as previously used by other workers, was investigated. Both light and temperature were found to affect the number of *A. fenestralis* entering the trays: it is concluded that although the trays do not accurately assess the *A. fenestralis* population of a bed, nor measure the rate of emergence from the bed, they are of value in assessing the relative effect of different control measures. In the filters the period of maximum abundance was during the spring, followed by a period of reduced numbers throughout the summer, with a recovery in the autumn giving relatively high numbers throughout the winter. Emergence of *A. fenestralis* from the filters is influenced by climatic factors, especially temperature and light; a diurnal variation in the rate of emergence was also observed. Because of this effect of temperature on emergence, the flies were only present above the filters in the warmer periods of the year; and because of the reduced numbers in the filters in the summer, the flies were only abundant above the beds during the spring and early summer and in the autumn. BHC applied as a water-dispersible powder was more effective than in the form of an emulsion. It was also most effective when used against the larval phase.

Introduction

The larva of *Anisopus fenestralis* (Scopoli, 1763) is an active grazer in some sewage filters, where it is beneficial in controlling the amount of bacterial and fungal film on the medium. An excessive amount of such growth is undesirable. Unfortunately the adult flies, because of their shade-seeking habits, enter buildings and dwellings on and near the works causing nuisances. They are attracted to food and have the obnoxious habit of depositing their egg masses in such moist shady places as on tooth-brushes, in sinks and in vegetable salads. On the works the aerial density of the flies at times may make work almost impossible. Because of its close association with sewage and food, *A. fenestralis* must be considered a potential danger to the health of the community besides causing both physical and mental distress, although no connexion between the fly and any disease has ever been proved. Filters as an environment for insects have been discussed by Lloyd (1935). Works treating domestic sewage usually have a balanced fly population consisting of several species

of psychodid and chironomid flies. Industrial trade wastes may restrict the fly fauna to few species, as at Huddersfield (Golightly, 1940). The presence of several species of flies results in interspecific competition for food and reduction in numbers of any one species (Lloyd, 1935; Lloyd, Graham & Reynoldson, 1940; Golightly, 1940; Lloyd, 1943*a*, *b*; Khalsa, 1948). The restriction of the fly fauna to few competing species may result in the abundance of one species from which nuisance might result. Complaints have been reported due to *Psychoda* spp. and to *A. fenestralis*. Although *A. fenestralis* is present in sufficient numbers to cause nuisance at several works (Khalsa, 1948), the Minworth Works of the Tame and Rea District Drainage Board is one of the few exceptions in having it as the dominant fly for the greater part of the year. At some sewage works it appears to be a relatively recent invader (Khalsa, 1948), but evidence points to its presence at Minworth for the past 30 years.

The Minworth Sewage Works

The works are situated in the Tame valley, 7 miles east of Birmingham. The three villages, Minworth, Curdworth and Water Orton, are all within the dispersal range of *A. fenestralis* breeding in the filters. The forty-two acres of filters are divided into six blocks having parallelogram-shaped filters and four smaller circular filters. The whole area is surrounded on all sides by trees; these act as a wind-screen and this no doubt favours the presence of *A. fenestralis*. The filters are above ground-level, and for the most part have open stone walls 6–7 ft. in height. The filtering medium varies in size throughout the works, but is mostly of 2–3 in. crushed stone in four of the blocks, and of round gravel of the same size in the other two blocks. Most of the experiments described in this paper were carried out on the last-mentioned blocks. Each of these blocks is divided into ten filters, on each of which the sewage is spread by two machines travelling backwards and forwards, removing sewage by syphon action from a trough running along the middle of the filter. For experimental purposes each filter was thus conveniently divided into four bays.

Only the final stages of treatment are carried out at Minworth, removal of suspended matter by sedimentation having been performed at the Saltley Works, $4\frac{1}{2}$ miles up the Tame valley. On arrival at Minworth a portion of the sedimented sewage receives partial purification with activated sludge before being applied to the filters in three blocks, including the two used in the experiments. The remainder of the sewage, after further sedimentation, is applied to the other three blocks. The sewage treated contains a high proportion of metallic and acid trade wastes, besides gas liquor. On four blocks the sewage is distributed from fixed nozzles projecting from pipes laid on top of the filter. The filter receives a continuous spray of sewage, but for several hours each day, depending upon the flow of sewage, the filters are rested. As already mentioned, the filters used in the experiments received sewage through travelling distributors which provided an intermittent dose on to each part of the filter about every 13 min. On the filters with fixed jets there develops every winter a thick surface fungal growth of *Fusarium* sp., and a fungus

referred to by Tomlinson (1941) as a new species of *Sepedonium*. This does not occur on the filters served by travelling distributors. A thin surface growth of unicellular Chlorophyceae appeared on these filters. Besides *A. fenestralis* the following macro-fauna was present in the filters: nematodes, *Lumbricillus lineatus* Mull., *Lumbricus* sp.; spiders and mites, *Achorutes subviaticus* Begn., *Tomoceros minor* Lubb., *Cercyon ustulatus* Preys., *Psychoda alternata* Say., *Psychoda cinerea* Banks., *Psychoda severini* Tonn., *Spaniotoma minima* Mg., *Leptocera fontinalis* Fln., *Spaziphora hydromyzina* Fln. and *Phygadeuon cylindraceus* Ruthe.

The life cycle of *Anisopus fenestralis*

Under optimum food conditions the full life cycle is completed in 121, 73, 50 and 39 days at 9, 13, 18·5 and 21° C. respectively (Tomlinson, 1943). In the filters, however, food may not always be plentiful. The effect of limiting the food supply available to the larvae was therefore investigated.

Culture methods. Nine egg masses of *A. fenestralis* were placed on pieces of filter-paper resting on wetted absorbent cotton-wool in Petri dishes. After hatching, twenty larvae were subcultured into similarly prepared dishes. The food used was activated sludge, a complex of bacteria, fungi and Protozoa, similar in nature to the film upon which the larvae graze in the filters. This food was prepared by centrifuging fresh activated sludge and sterilizing by boiling. The resultant paste was then applied to the filter-paper in the Petri dish. The food was applied frequently; at first in small quantities but increasing as the larvae grew. One set of three cultures was provided with excess food, a second set with half this amount and a third with one-tenth. This was achieved by preparing the sludge paste from the appropriate dilution of the fresh sludge. The pupae and adults were cultured in 2 × 1 in. glass tubes, in the bottoms of which moist wads of absorbent cotton-wool were placed; when required sucrose was added to these tubes. The cultures were incubated at approximately 19° C. and examined daily, the date of eclosion, pupation and emergence being noted. Some adults were provided with sucrose, others received no additional food. To determine the effect of starvation on the size of the pupae five from each set, chosen at random, were measured. The results are summarized in Table 1. The results show that by limiting the food available to the larvae this

Table 1. *The effect on the life cycle of limiting the food supplied to the larvae of* Anisopus fenestralis

Food provided (units)	10	5	1
Duration of larval phase (days)	30 ± 0·65	34 ± 0·77	69 ± 2·7
Duration of pupal phase (days)	8·6	8·75	8·5
Duration of adult phase (not fed) (days)	9 ± 0·55	7 ± 0·34	5·3 ± 0·44
Duration of adult phase (fed on sucrose) (days)	21	20	47
Average length of pupae (mm.)	8·4	8·6	5·1

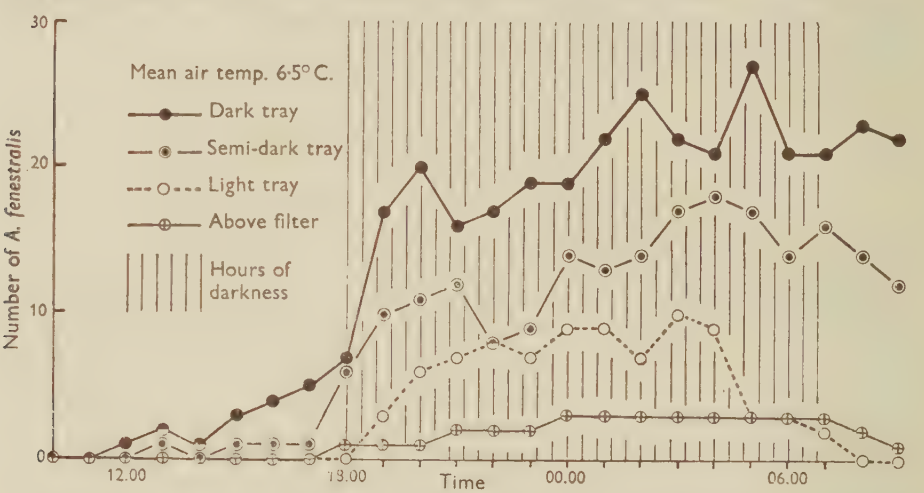
phase is lengthened, the mean size of the pupae is reduced and the duration of the adult phase, when not fed, is shortened. The duration of the adult phase is considerably increased by feeding with sucrose. Other work, not reported here, also indicated that the pre-oviposition period was also lengthened when the female adult was allowed to feed on sucrose.

Methods of assessing *Anisopus fenestralis* populations in and above the filters

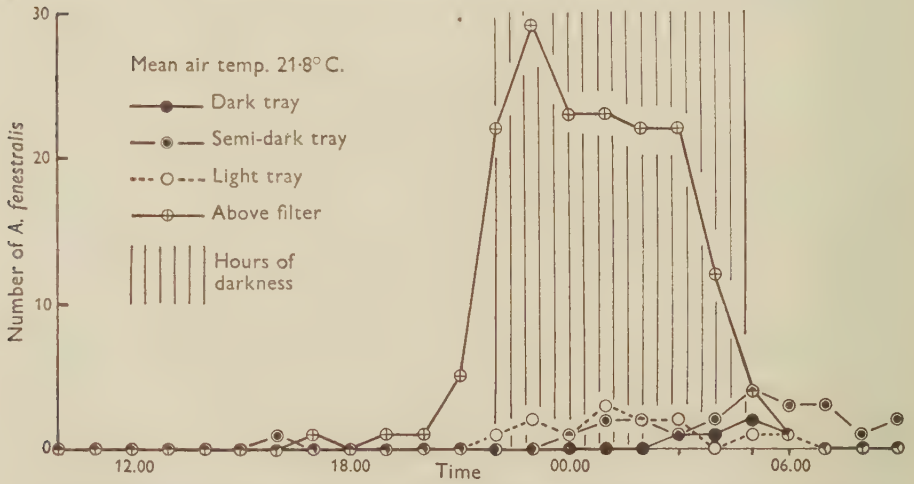
Tray traps. These traps were introduced by Tomlinson & Stride (1945) to investigate the fly population of sewage filters. The trap consists of a metal tray 1 ft. square and $2\frac{1}{2}$ in. deep, which is inverted over the filter. Periodically the tray is removed and placed in the inverted lid. The trapped flies are then chloroformed and counted. The tray trap has been widely used in the control experiments, and in assessing *A. fenestralis* populations and incidence (Tomlinson & Stride, 1945; Tomlinson & Jenkins, 1947; Tomlinson & Muirden, 1948; Tomlinson, Grindley, Collett & Muirden, 1949; Khalsa, 1948; McLachlan & Hogg, 1948; Jenkins, Baines & Hawkes, 1949). The exact significance of the numbers of flies found in these trays has never been defined, though reference is made to 'flies which had migrated into the trays' (Tomlinson & Stride, 1945) and 'flies emerging from that part of the filter covered by the trap' (Tomlinson & Jenkins, 1947). Investigation into this method of trapping was therefore considered necessary. Khalsa (1948), working on *A. fenestralis* populations, noted differences between results obtained using tray traps and other trapping methods. McLachlan & Hogg (1948), in work on *Psychoda* spp., found that by modifying the tray by fitting 'a fine gauze top to maintain as far as possible the normal environment by letting in the light and spray' no flies were trapped. If it is intended to measure the emergence of flies from the filter, then the effect of the different environment created over that area of filter covered by the trays should be considered. Experiments were carried out to investigate the effect of light and temperature on the numbers of *A. fenestralis* trapped.

Effect of light. Three types of trays were used: (*a*) the standard tray trap, (*b*) a tray of similar dimensions but having a 4 in. square Perspex window fitted in the bottom and known as the semi-dark tray; and (*c*) a similar tray but having the 1 ft. square bottom made entirely of Perspex and known as the light tray (Pl. 14). These trays were inverted over the filter, and at hourly intervals they were removed in order to count the number of *A. fenestralis* in each tray. The trays were then returned to the filter without disturbing the trapped flies. A measure of the number of *A. fenestralis* above the filter at different times throughout the 24 hr. was obtained by noting hourly the number of flies observed on three 1 sq.ft. areas facing different directions. Hourly readings of the temperature of the three trays and of the air was taken. The numbers of *A. fenestralis* found hourly in three types of tray and on

unit area above the filter were plotted together with the air temperature. The experiment was repeated at different times of the year; the results of the October and June experiments, which illustrate the effect of diverse climatic conditions on



Text-fig. 1. Relative numbers of *A. fenestralis* found hourly in three types of tray and on unit area above the filter. October 1948.



Text-fig. 2. Relative numbers of *A. fenestralis* found hourly in three types of tray and on unit area above the filter. June 1949.

the tray trapping, are shown in Text-figs. 1 and 2. Text-fig. 1 shows that during the hours of daylight in October most flies were trapped in the dark trays and least in the light ones. At dusk there was an increase in the rate of entry of flies into all

trays, whereas at dawn the flies were repelled from the light trays. Few flies were observed above the filter and these only in the hours of darkness. By contrast, the results in June (Text-fig. 2) showed that although few flies entered the trays, considerably more were observed above the filter. From these results it is concluded that light tends to inhibit the migration of *A. fenestralis* into the trays. Also the number of *A. fenestralis* trapped into the surface trays is no true measure of the emergence of the fly from the filter. The effect of light on the results obtained with the light and semi-dark trays is understandable, but its effect on the results with the dark trays can only be explained by assuming that *A. fenestralis* entered the trays, seeking shelter, from surrounding areas exposed to light. The difference between the June and October results could be explained by assuming that when conditions were not favourable for emergence the flies took shelter in the trays,

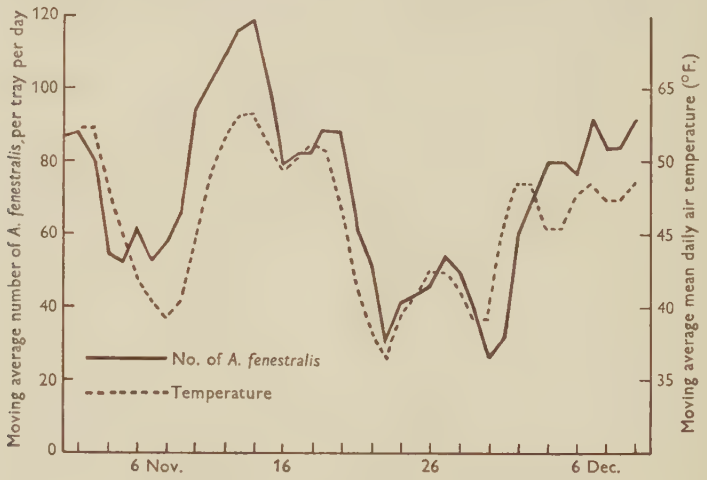
TABLE 2. *Comparison between the mean number of flies trapped in the inner and outer trays of squares of sixteen trays*

Count in	Exp. no.	Mean count in inner trays	Mean count in outer trays	Difference between mean value for inner and outer trays
December 1948	1	18 ±2·27	25 ±3·25	+ 7 ±3·95
	2	13 ±1·0	19 ±2·3	+ 6 ±2·5
	3	22 ±1·9	37 ±2·5	+15 ±3·15
	4	39 ±3·3	58 ±5·5	+19 ±6·5
	5	31 ±2·0	40 ±5·0	+ 9 ±5·4
	6	20 ±3·4	52 ±3·8	+32 ±5·1
June 1949	1	2·8 ±0·41	2 ±0·36	− 0·8 ±0·55
	2	14·5 ±3·57	14·6±1·4	+ 0·1 ±3·85
	3	4·5 ±1·42	5·4±0·41	+ 0·9 ±1·47
	4	3·25±1·4	3·4±0·67	+ 0·15±1·56
	5	2 ±0·18	3·4±0·66	+ 1·4 ±0·68
	6	2 ±1·2	1·6±0·42	− 0·4 ±1·27

but when conditions were favourable they did not seek shelter. To test this hypothesis a square of sixteen trays was arranged on the filter, and the numbers of *A. fenestralis* trapped in the inner trays compared with those trapped in the outer ones. This experiment was made in December when few flies were observed above the filters, and repeated in June when conditions favoured emergence. The results of six sets of counts for each experiment are given in Table 2. In December counts more flies were found in the outer trays in all six experiments, the difference being statistically significant in four out of the six counts. If the tray trapped the flies emerging from 1 sq.ft. of the filter covered by it, then there should be no difference between the numbers trapped in the inner and outer trays. The larger number found in the outer trays indicates that *A. fenestralis* migrates into the trays from surrounding areas when conditions do not favour emergence. The inner trays, being protected by the outer trays, did not have the opportunity of attracting flies from the surrounding area. In the June counts, when conditions favoured emergence,

in only four out of six cases did the number of flies in the outer trays exceed those in the inner ones, the difference being slight and significant in only one case. It was concluded that *A. fenestralis* only sought shelter of the trays when climatic conditions were adverse to emergence.

Effect of temperature. To investigate the effect of temperature on the migration of *A. fenestralis* into the trays the mean number found daily in eighteen trays was plotted over a period of 40 days in November and December 1948. The mean daily temperature, as measured by the average of the maximum and minimum temperatures for the 24 hr. period, were also plotted; day-to-day irregularities in the sampling being smoothed out by applying a 3-day moving average. From the graphs (Text-fig. 3)



Text-fig. 3. Relationship between numbers of *A. fenestralis* found daily in trays, and the mean daily temperature.

it is seen that for this period there was a close correlation between the number of *A. fenestralis* found in the trays and temperature. By plotting the mean daily temperature and the mean number of *A. fenestralis* found daily in the trays from non-treated areas throughout 1949, it was found that this correlation only held in the colder periods of the year when emergence from the filters was slight.

Discussion on tray traps. The above results suggested that the number of *A. fenestralis* trapped in the trays is a measure of the number at the surface of the filter seeking shelter when climatic conditions discourage emergence. Light and temperature influence the number of flies at the surface; in daylight and in cold weather the flies tend to keep to the warm dark depth of the filter, and therefore only few enter the trays. Increase in temperature and decrease in light intensity results in larger numbers being present at the surface, and consequently the numbers trapped increase. When conditions favour emergence, however, although there may be large

numbers at the surface few enter the trays. Trapping by these trays does not therefore give a true measure of emergence nor does it give comparative results for the numbers of flies in the filters at different seasons. The emergence is better measured by the use of traps above the filter, such as the canister trap (Tomlinson & Stride, 1945) or by sticky Perspex cross, as described below; estimates of flies in the filters may be obtained by counting larvae and pupae in the filter. In tests on control measure, however, by careful choice of treated and control areas, comparative counts using tray trapping may be obtained. Other methods of assessing fly populations of filter beds have previously been used. The jar trap, as used by Lloyd (1935), was found difficult to manipulate in large numbers and impracticable on some filters because of the machinery distributing the sewage being too near the surface. Methods employing sticky traps have been used in other fields to study aphis migration (Broadbent, 1948). The use of 1 sq.ft. sheets of sticky Perspex placed immediately over the filters was tried. Although these trapped large numbers of chironomid flies, few *A. fenestralis* were trapped and condensation interfered with the sticky material. To assess the aerial density of *A. fenestralis* above the filters the Perspex 'cross' was designed (Pl. 15). This consists of four vertical sticky sheets of Perspex, each approximately 6 in. square, facing four different directions at right angles to one another. By this arrangement the wind direction, which would influence the flies trapped on a single surface, does not affect the mean number trapped. The cross was mounted 4 ft. above the filter on a post carried on a mechanical distributor.

Seasonal incidence of *Anisopus fenestralis* and some factors influencing emergence from the filters

In studying the seasonal incidence of *A. fenestralis*, a distinction was made between the incidence in and above the filters. Factors influencing the egress of the flies were also considered. Tray traps were used to assess the incidence in the filter, although it was realized that the results might be influenced by factors previously referred to. The mean of six daily counts on traps set on areas which had not been treated with benzene hexachloride (BHC) was taken as indicating the frequency of *A. fenestralis* in the filter; the average daily temperature in the trays was also found. The mean number of *A. fenestralis* caught daily on the four surfaces of the 'cross' was taken as a measure of the aerial density above the filters; the average daily air temperature above the filter was also noted. The results are shown in Tables 3 and 4. These results of assessment by tray trapping indicate that the period of maximum abundance of *A. fenestralis* in the filter is during the spring. Throughout the summer months the numbers are reduced. An increase occurs in the autumn, and large numbers are found throughout the winter. Above the filter the fly was present in large numbers only during warm periods when the mean temperature exceeded 52° F. Tomlinson (Discussion on Jenkins *et al.* 1949) reported that below 54° F. *A. fenestralis* showed little flight. During the warmer periods there appear to

be two periods of abundance, one in June and another in the autumn. The decreased numbers caught on the aerial trap throughout the summer coincided with the reduced numbers found in the tray traps. Emergence of the flies from the filter is influenced by temperature, although other factors such as wind velocity are also considered important. Previous experiments showed that the rate of emergence increased at dusk, which suggests that light is also a contributory factor. This phenomenon might be due to a natural diurnal rhythm of activity, as demonstrated for several mosquitoes (Bates, 1949).

TABLE 3. *The incidence of* Anisopus fenestralis *in the filter as measured by tray trapping*

Month	No. of days each month when mean tray count exceeded 25	50	75	100	Mean tray temp. (° F.)	Temp. range
Jan.	15	5	1	0	42	34–49
Feb.	15	5	1	0	44	37–51
Mar.	12	8	3	1	42	34–51
Apr.	18	10	2	1	51	43–63
May	10	1	0	0	57	44–73
June	5	0	0	0	63	53–76
July	0	0	0	0	65	51–77
Aug.	1	0	0	0	62	56–70
Sept.	2	0	0	0	62	58–70
Oct.	6	0	0	0	55	40–68
Nov.	9	1	0	0	46	41–50
Dec.	13	7	2	1	42	38–48

TABLE 4. *The incidence of* Anisopus fenestralis *above the filter as measured by the sticky 'cross' method*

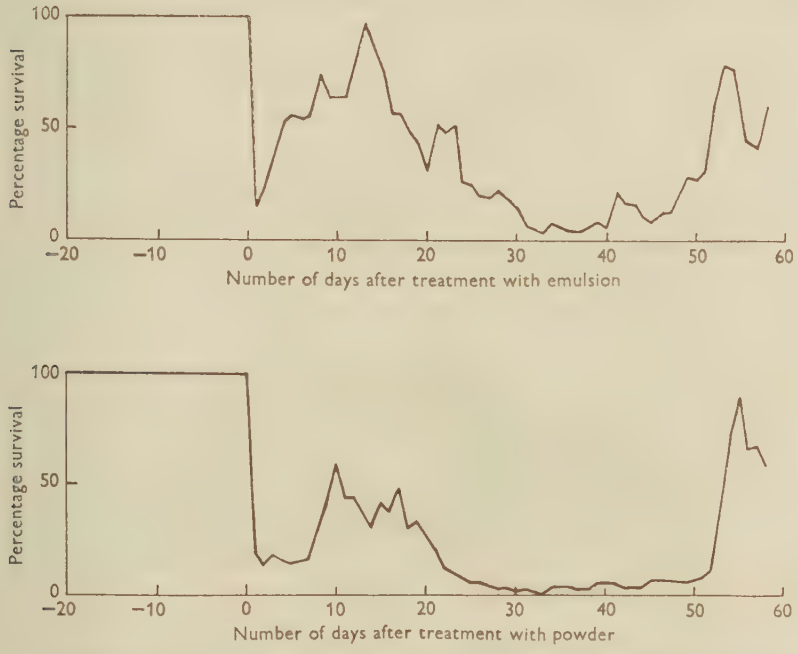
Month	No. of days each month when numbers caught on 'cross' exceeded 100	200	400	600	800	1000	Mean air temp. (° F.)	Temp. range
Jan.	0	0	0	0	0	0	42	35–50
Feb.	0	0	0	0	0	0	43	32–51
Mar.	4	3	1	0	0	0	41	34–55
Apr.	7	4	1	1	0	0	50	40–57
May	14	12	5	0	0	0	53	46–58
June	21	16	5	4	2	2	60	53–72
July	12	6	1	0	0	0	64	54–75
Aug.	11	5	0	0	0	0	62	55–70
Sept.	17	11	1	0	0	0	62	56–60
Oct.	11	5	4	0	0	0	55	44–66
Nov.	0	0	0	0	0	0	45	38–50
Dec.	0	0	0	0	0	0	42	35–47

EXPERIMENTS IN CHEMICAL CONTROL

Attempt to control the number of *A. fenestralis* at Minworth was attempted in 1933, when bleaching powder, salt and gas liquor were applied to the filters. Later, the insecticides DDT and BHC were used with success (Tomlinson & Jenkins,

1947; Tomlinson & Muirden, 1948; Tomlinson *et al.* 1949). In continuation of these experiments the effect of BHC, used as a water-dispersible powder and as an emulsion, were compared.

Method of application. The BHC emulsion or dispersible powder required for each filter was diluted with sewage and siphoned for a period of 1 hr. into the sewage being supplied to the filter, which was not dosed with sewage for 6 hr. before treatment and for 17 hr. thereafter. All applications were at the rate of 1·3 lb. of the active γ-isomer per acre. In the autumn of 1948 the effect of a single

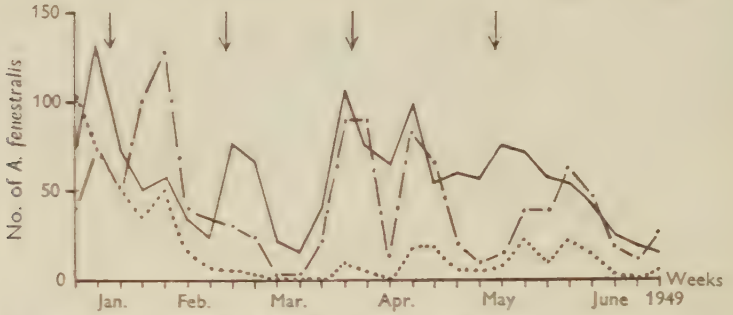


Text-fig. 4. Effect of single application of BHC as an emulsion (above), and as a water-dispersible powder (below).

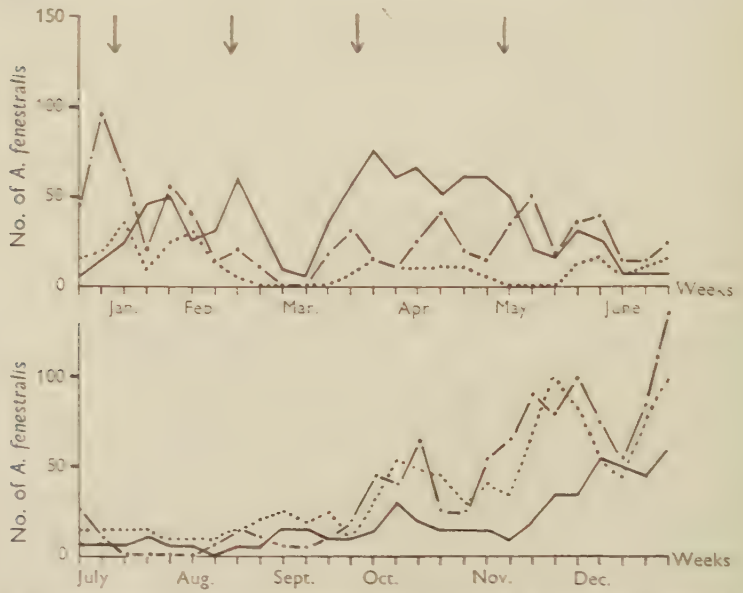
application to two blocks of filters was studied by tray trapping on four filters, two of which were treated with dispersible powder and two with the emulsion. Half of each of the four filters served as untreated controls. Three tray traps were set on each of the treated and untreated areas. Daily counts of *A. fenestralis* were made on the 20 days before and on the 60 days after the application. The difference between the mean numbers of *A. fenestralis* trapped daily on control and treated filters was expressed as a percentage of the numbers trapped on the control filters. The percentage reduction is shown in Text-fig. 4, which illustrates the effect of the treatment for 60 days following the application of BHC.

During the first 6 months of 1949, four applications were made. On each block

six tray counts were made daily on untreated control areas and on areas treated with the emulsion and with the dispersible powder. To investigate the possibility of any



Text-fig. 5. Comparative effect of treatment with BHC as an emulsion, and as a water-dispersible powder. January–June 1949. ——, not treated; —·—·, treated with BHC as emulsion; ·····, treated with BHC as a water-dispersible powder; ↓, times of treatment.



Text-fig. 6. Comparative effect of treatment with BHC as an emulsion, and as a water-dispersible powder (1949). ——, not treated; —·—·, treated with BHC as emulsion; ·····, treated with BHC as a water dispersible powder; ↓, times of treatment.

delayed effect of the treatment the counts on one block were continued throughout the year. The weekly means of the six daily fly counts on each area are shown in Text-figs. 5 and 6. The results of the single applications in 1948 showed that for

60 days following treatment with emulsion a 63% reduction was effected, compared with a figure of 79% for the powder. The results of the four applications in 1949 showed that BHC as an emulsion effected a reduction of only 18·5% over a 6-month period compared with 70% by the powder. The better control effected in the autumn may be explained by the fact that at that time the film was relatively thin compared with the amount present when the applications were made in 1949. This effect has been previously reported (Discussion on Tomlinson *et al.* 1949). The graphs in Text-fig. 6 show that treatment at the rates used did not produce control over a prolonged period. For the second half of the year, when no treatment was given, the areas which had previously been treated had a larger *A. fenestralis* population. A probable explanation is that in the untreated areas the amount of food became limiting, whereas in the treated areas, because of reduced grazing activity, the food material was allowed to accumulate; however, measurements of the amount of film were not made.

The percentage control has been given as the mean for the whole period following treatment, but as shown in the graphs in Text-fig. 4 this varies during the period. The graphs show that there is an immediate reduction due to the death of adult flies in the filter, followed by a recovery period of 15 days due to the emergence of adults from pupae unaffected by the treatment. This immunity of the pupae might be due to their greater resistance or to their ecological position in the drier regions of the filter, where they are least likely to receive the insecticide mixed with the sewage. A second reduction then occurred due to the effect of the insecticide on the larvae in the filters at the time of the treatment. This persisted for approximately 30 days. From these results it is concluded that BHC is most effective when used against the larval stage of *A. fenestralis* and least effective against the pupal stage. The toxicity of BHC on various stages of *A. fenestralis* has been reported by Lloyd (Discussion on Tomlinson *et al.* 1949).

Discussion

The incidence of *A. fenestralis* in the filters can be correlated with temperature and food factors. Culture work has shown that both temperature and food are important in determining the length of the larval phase. In the filters the relative effects of these two factors may vary throughout the year; for in the winter, when food is plentiful, temperature will be the limiting factor, whereas in the summer, due to increased grazing activity, food may become the limiting factor. In the spring, when food is still plentiful and temperatures are rising, conditions are at an optimum for larval development. The shorter the larval phase, the greater the number of generations produced in a given period and hence the larger the adult population. Lloyd (1941, 1943*b*) has demonstrated the effect of temperature on the seasonal rhythm of *Spaniotoma minima* and *Psychoda alternata*. A similar rhythm for *A. fenestralis* was indicated at Minworth, and it is considered that this rhythm would also be influenced by the food factor. On this basis, a spring increase would be

expected, followed by a decrease throughout the summer. This is supported by the incidence of *A. fenestralis* at Knostrop Sewage Works (Khalsa, 1948) and the results obtained at Minworth. The effect of food on the incidence of *Psychoda* spp. has previously been reported (Fair, 1934; Golightly, 1940).

The results of the author's experiments on chemical control showed BHC to be most effective as a larvicide. To be used most effectively the treatment should coincide with the larval phase of the rhythm. If the insecticide is applied only when there is an immediate danger of nuisance from adults, it is likely that there will be a large pupal population in the filter which would survive the treatment. Apart from any natural rhythm, an artificial one is induced by the survival of pupae on treatment and by examination of the filters, later applications can be given at the appropriate times. A nuisance is only likely to arise when there are both large numbers of *A. fenestralis* in the filters and climatic conditions favour emergence. Such conditions usually occur in late spring and early summer, and any potential nuisance then should be averted by treatments against the larval phases of these generations in March and April. By this method the beneficial feeding activities of the larvae is retained as long as is compatible with the prevention of nuisance. In assessing the effect of control measures it is considered that tray trapping gives valid results, though, because of interference by climatic changes throughout the year, this method does not give a true measure of the incidence of the fly in or above the filters nor does it measure the rate of emergence of the flies from the filter. A measure of the incidence of the fly is given by counts of larvae and pupae found in the filter, whereas the rate of emergence may be measured by aerial trapping.

This work was carried out in connexion with the fly-control programme of The Birmingham Tame and Rea District Drainage Board, and forms part of a thesis submitted by permission of F. C. Vokes, B.Sc., M.I.C.E., Engineer to the Board, for the degree of M.Sc., of Leeds University. I wish to express my gratitude to Dr S. H. Jenkins, Board's Chemist, for his guidance and interest throughout the experiments, and to thank Prof. E. A. Spaul and Dr Ll. Lloyd for advise and criticism offered. My thanks are also due to members of the Laboratory staff and workmen who assisted me in the daily task of fly counting.

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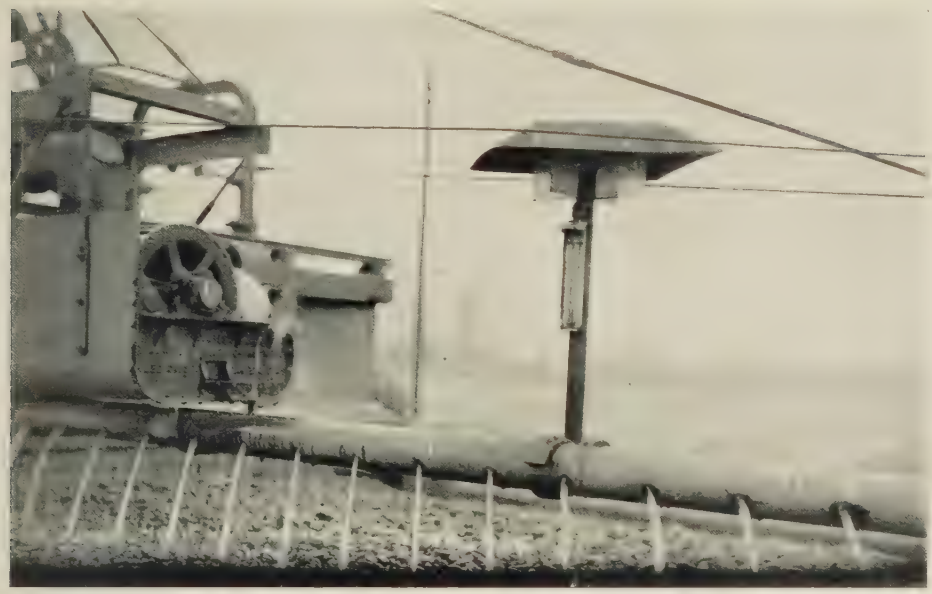

Fig. 1

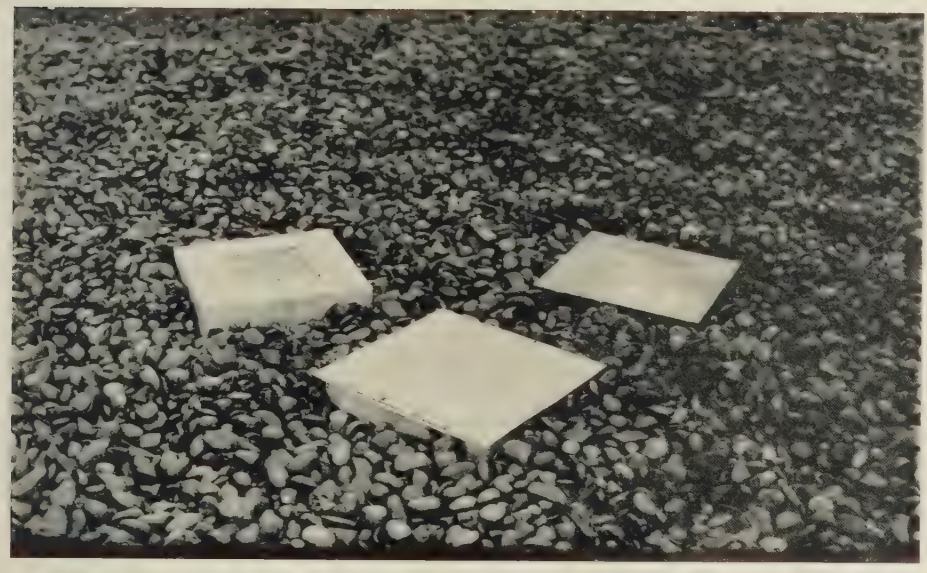

Fig. 2

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EXPLANATION OF PLATE 15

Fig. 1. The Perspex 'cross' trap for measuring the incidence of *A. fenestralis* above the filters.

Fig. 2. The three types of tray trap used to determine the effect of light on the trapping of *A. fenestralis* in surface trays.

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POPULATION STUDIES ON *HYLOBIUS ABIETIS* (L.) (COLEOPTERA, CURCULIONIDAE)

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(With 3 Text-figures)

Populations of third-stage larvae, pupae and adults of *Hylobius abietis* in the stumps of pine trees, *Pinus sylvestris* L., have been assessed. On a felled area the relative numbers of adults have been obtained by trapping; the most reliable estimate of absolute numbers was obtained from the stump sampling data.

Introduction

The biology of *Hylobius abietis* described by Munro (1928) is briefly as follows. The first weevils to appear in spring are old ones which have spent one or more summers in the open. Later they are reinforced by others which matured too late the previous year to emerge from the stumps in which they developed, and passed the winter in the pupal beds in the wood. These in their turn are followed by newly emerged beetles which passed the previous winter as larvae in the stumps. The build-up of mass populations requires a large amount of suitable breeding material such as is presented in freshly exposed pine stumps.

Economically *Hylobius* is important because the adults feed on young pine trees, causing stunted growth in seedlings and preventing the formation of straight poles. No quantitative studies appear to have been made upon *Hylobius* populations, and the present work was done to provide such data.

The area chosen was at Windmill Stem, Bagshot Woods, Surrey, where 40-year-old pine trees had been felled over three different periods, commencing October 1947, January 1948 and July 1948. In Fig. 1 the areas felled during these respective periods are labelled *A*, *B* and *C*. Area *A* had been clear-felled and used as a roadway for hauling out timber; in areas *B* and *C* mother trees had been left to provide seed for natural regeneration. Area *C* differed from the other two in that the ground had been bulldozed in an attempt to encourage natural regeneration by removal of the thick surface layer of dead pine needles, and by the loosening of the soil.

Stump sampling for larvae

Adult weevils were not evident when work began early in April 1949; it was therefore decided to investigate the third-stage larvae which persist throughout the winter in the stumps.

Before any serious stump sampling could begin, it became necessary to find some

visual method of judging stump age. One-year-old stumps, i.e. those exposed in 1948, usually had the dried and cracked area on the surface restricted to a central circle of about 2 in. diameter, the remainder being encrusted with a pale green hardened resin. The bark of the stump was also usually closely adherent. Two-year-old stumps, on the other hand, had usually no encrustation and were cracked all over the surface, whilst the bark had begun to peel away from the wood. In 3-year-old stumps drying had proceeded much further, so that the wood could be readily broken away.

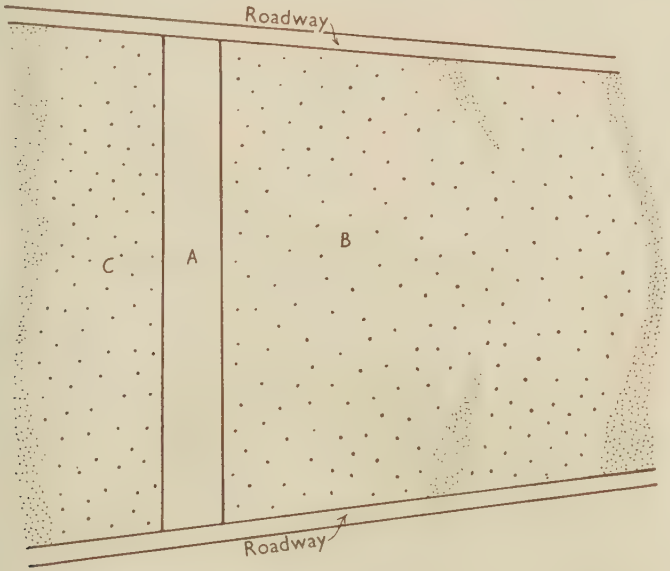

Fig. 1. Plan of the felled area, Windmill Stem. ∴∴, mother trees; ▤, young pine plantations.

TABLE 1. *Mean distribution of larvae along the roots of pine*

	Section of root measured from stump					
	1st ft.	2nd ft.	3rd ft.	4th ft.	5th ft.	6th ft.
Total larvae (15 roots)	58	37	23	7	1	0
Means	3·87 ± 1·08	2·47 ± 0·91	1·53 ± 0·76	0·47 ± 0·03	0·07 ± 0·02	0

In sampling the stumps the top 9–12 in. of soil was cleared and the roots exposed laterally in a circle of about 4 ft., in which area most of the larvae occurred. They were chiefly in chambers in the bark of the bole and in the surface wood of the roots.

Comparison of larval populations in 1- *and* 2-*year-old stumps.* Areas *B* and *C* were each divided into twelve plots, 50 × 30 yd., and area *A* into six plots of the same

size giving the five blocks C_2, C_1, A, B_1 and B_2 shown in Table 2. Areas B and C were split longitudinally to compare the larval populations in the inner strips B_1 and C_1 with those in the outer strips B_2 and C_2, as each inner strip had been cleared a short time before the respective outer one. Three stumps of one age group in each plot were selected at random for sampling in the manner described. Results of the sampling done from 21 April until 27 May 1949 are shown in Table 2.

TABLE 2. *Larval populations in stumps (means of three samples)*

Area C (felled July 1948)		Area A (felled October 1947)	Area B (felled January 1948)	
C_2	C_1		B_1	B_2
32·66	34·66	N.S.	41·33	28·00
20·00	46·33	2·33	73·00	46·33
29·66	32·00	1·33	51·66	28·66
34·00	37·33	0·66	59·00	50·00
22·66	34·00	1·33	40·00	28·66

TABLE 3. *Analysis of variance of data from 1-year-old stumps*

Factor	Sum of squares	Degrees of freedom	Variance	F by calculation	Table of F $P=0·05$
Total	3156·98	19	—	—	—
Area	760·64	1	760·64	8·13	4·49
Subarea	876·06	1	876·06	9·37	4·49
Interaction area and subarea	24·06	1	24·06	—	—
Error	1496·22	16	93·51	—	—

Mean infestations: area B, 44·64;
area C, 33·23; S.D. ($P=0·05$), 9·17.

Mean infestations: areas B_1+C_1, 44·93;
areas B_2+C_2, 31·96; S.D. ($P=0·05$), 9·17.

The difference between infestation of the older stumps on area A and the newer ones on areas B and C is apparent; it is not certain whether this difference is due to the unsuitability of the older stumps for large populations of larvae, or whether the ovipositing female weevils were attracted more strongly in 1948 to the freshly cut stumps. Laboratory experiments show that all pre-adult stages are readily susceptible to desiccation, so field differences in infestation were probably due to the adverse environment of the drier older stumps.

Differences between larval populations in 1-year-old stumps. Table 3 gives an analysis of variance of 1-year-old stumps. Comparisons have been drawn between infestation on areas B and C (area sum of squares), and between those on the inner strips B_1+C_1 and the outer strips B_2+C_2 (subarea sum of squares).

The January stumps (area B) were probably more infested than the July stumps (area C), because they had been longer exposed to egg laying. Similarly, during

felling operations in 1948 the trees on the more heavily infested inner strips B_1 and C_1 were cut down before those in B_2 and C_2. It was noted in the laboratory that female weevils laid at a slow but steady rate throughout the season.

Comparison of larval populations in stumps and roots. Separate counts were made of the larvae in the bole of the stump and in the roots (Table 4). The roots having the larger surface and hence food supply support the larger population.

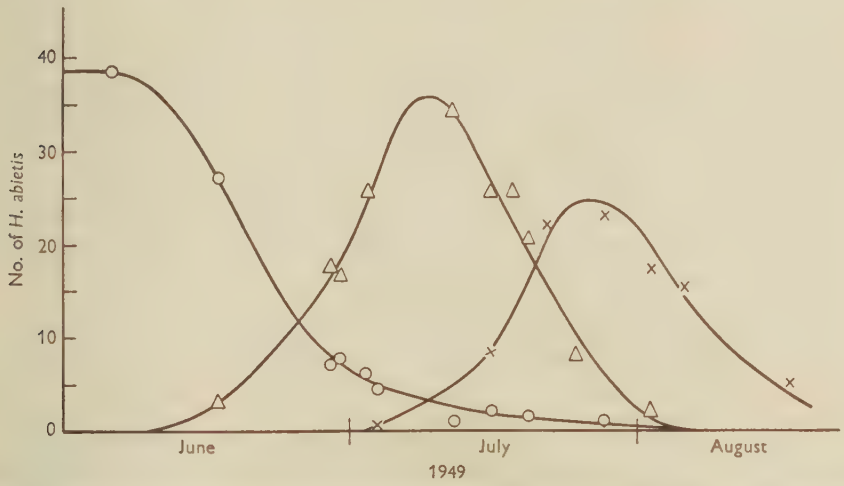

Fig. 2. Average numbers of third-stage larval, pupae and adults of *H. abietis* per stump. ○, larvae; △, pupae; ×, adults.

TABLE 4. *Larval numbers in stumps and roots (means of three samples)*

Area *B*		Area *C*	
Stump infestation	Root infestation	Stump infestation	Root infestation
16·66 ± 0·76	27·33 ± 3·91	13·63 ± 1·03	19·03 ± 2·06

Mean of combined stump infestation, areas $B+C$ = 15·14 ± 0·71.
Mean of combined root infestation, areas $B+C$ = 23·18 ± 2·36.
Difference between the means of the combined infestations = 8·04.
(Significant at $P = < 0·01$)

DEVELOPMENT OF *HYLOBIUS* IN THE STUMPS

At the beginning of April 1949, the third-stage larvae were in pupal chambers cut in the bark of the stump and in the outer wood of the roots. Pupae were found from 16 June until 3 August, and the first adults appeared on 2 July. Sampling ceased on 17 August.

On each sampling date at least two stumps were recorded. Fig. 2 shows the data from the 1-year-old stumps, the mean larval infestation of 38·32 being obtained from Table 4.

The peak pupal infestation (34·75) is only slightly below that of the mean larval infestation, 38·32, indicating a very low death-rate in the late third larval and early pupal stages. Pupation occupied approximately 3 weeks. Empty pupal chambers were first observed on 19 July, emergence from the stumps being the main reason for the great difference in the height of the pupal and adult peaks, although there was slight mortality.

Mortality of *Hylobius* in the stumps

Munro (1929) concludes that fungal and bacterial diseases are unimportant in regulating numbers of *Hylobius*. He found only one larval parasite, closely allied to *Bracon hylobii* Ratz, acting as a check, but it sometimes destroyed 30% of the larvae in a locality. No parasites were found in the Bagshot Woods area.

Table 5 shows mean numbers of dead and live stages of *Hylobius* per stump recorded in this investigation. The empty pupal chambers represent adults which have emerged from the stumps, and these weevils have been included in the totals for the purpose of estimating percentage mortalities.

Table 5. *Average numbers of dead and live stages of* Hylobius *per stump (means of two or more samples)*

	Third-stage larvae		Pupae		Adults		Empty pupal	
Date	Alive	Dead	Alive	Dead	Alive	Dead	beds	Total
25. iv. 49	34·0	0·6	0	0	·0	0	—	34·6
28. iv. 49	37·3	1·0	0	0	0	0	—	38·3
17. v. 49	29·6	1·6	0	0	0	0	—	31·2
16. vi. 49	27·0	0	3·0	0	0	0	—	30·0
28. vi. 49	7·0	0	17·5	0	0	0	—	24·5
29. vi. 49	7·6	0	17·2	0	0	0	—	24·8
2. vii. 49	6·1	0	25·5	0	0·1	0	—	31·7
3. vii. 49	4·4	0	20·8	0	0·6	0	—	25·8
11. vii. 49	1·0	0	34·0	0	1·0	0	—	36·0
15. vii. 49	2·0	0	25·6	0	8·3	0	—	35·9
19. vii. 49	1·5	0	20·5	0	7·5	0	1·0	30·5
21. vii. 49	3·0	0	27·0	0	22·0	0	0	52·0
24. vii. 49	0	0	8·0	0	16·0	0	5·0	29·0
27. vii. 49	0·5	1·5	1·0	0	20·5	0	8·0	31·5
3. viii. 49	0	2·0	2·6	1	17·3	1	7·6	31·5
10. viii. 49	0·5	5·5	0	0	13·0	0	38·0	57·0
17. viii. 49	0	0	0	0	5·0	0	23·0	28·0
Total	161·5	12·2	202·7	1·0	111·3	1·0	82·6	572·3

Percentage mortality: third-stage larvae, 7·56; pupae, 0·18; adults, 0·05.

The calculated figure, 7·72 for combined larval and pupal mortalities, agrees fairly closely with the figure 9·31 obtained from the curves in Fig. 2.

Fluctuations in numbers of adults on the open area

Population changes were followed by using a modified form of Grohmann trap from 22 April until 17 August 1949. Twenty-two traps, twelve on area *C* and ten on area *B*, were sited at random. Each consisted of a pit, $3 \times 3 \times \frac{1}{2}$ ft., containing

two fresh pine logs covered with fresh pine brush. The logs were renewed about every 6 weeks, and the pine brush when and where required.

Fig. 3 shows the rapid increase in population to a peak on 21 May, when fifty-nine weevils were caught, and then a steady decline until 11 June. A sharp rise starting 12 July reached a maximum of 145 on 29 July. From then until the records closed on 17 August the catches showed great fluctuations. The sharp drop from 29 July until 2 August coincided with very heavy rains and flooding of the traps.

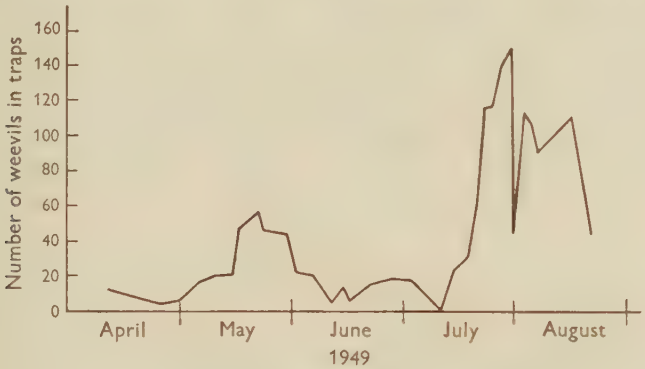

Fig. 3. Population fluctuations of *H. abietis*.

Examination of the reproductive organs of female weevils caught from the beginning of emergence up to 29 May showed fully developed organs and sperm in the spermatheca; these therefore were old weevils which had hibernated throughout the winter in the soil. Three live weevils and one dead one were found on 8 May, 1 in. below the soil surface beneath a young pine tree. Weevils which had not yet copulated and having undeveloped ovaries were first found on 16 June; these weevils had matured too late in 1948 and had passed the winter in pupal beds in the stumps. Emergence of the main brood, as shown by dissection, began in the second week of July.

Estimation of the numbers of adults on the felled area

Jackson (1939, 1948) caught and marked tsetse flies, *Glossina morsitans* Westwood, at regular intervals and then corrected the recaptures as if 100 had been marked and the same number caught on every recapture date. This gives a series of corrected recapture figures, y_1, y_2, y_3, etc., from which is found the average ratio of each value of y to the one preceding it; this is denoted by r. The corrected recapture value, y_0, for the date when the marked insects were released is obtained by the formula

$$y_0 = \frac{y_1 + y_2 + \ldots + y_{k-1}}{r} - (y_1 + y_2 + \ldots + y_{k-2}),$$

where k equals the number of recapture dates.

The above technique is called the 'positive' method. In the 'negative' method of Jackson the population on any particular date is obtained from recaptures of flies marked in previous catches.

Experiments with *Hylobius* were started on 19 July, marked insects being trapped and released every 2 days, and ended on 31 July. A different colour was used on each marking date. As the population was increasing due to adult emergence from the stumps it was necessary to make the releases as large as possible, so catches from the modified Grohmann traps were supplemented with weevils caught on fresh pine logs laid in a groove on the ground.

TABLE 6. *Data on marking experiments with* Hylobius abietis

Marked releases	Trap catches	Numbers of marked recaptures on dates stated							
		19. vii. 49	21. vii. 49	23. vii. 49	25. vii. 49	27. vii. 49	29. vii. 49	31. vii. 49	
83	—	1							
192	56	1	21. vii. 49						
146	112	2	8	23. vii. 49					
141	113	0	5	0	25. vii. 49				
195	133	0	3	0	1	27. vii. 49			
185	145	0	1	2	0	0	29. vii. 49		
98	98	0	0	0	1	0	1	31. vii. 49	

An estimate of the population on 21 July 1949 using the data of Table 6 is given below:

Positive method for estimation of the population on 21 *July* 1949

$$k=4;$$

$$y_1=\frac{3\times 10^4}{192\times 112}=1{\cdot}395; \quad y_3=\frac{3\times 10^4}{192\times 133}=1{\cdot}175;$$

$$y_2=\frac{5\times 10^4}{192\times 113}=2{\cdot}305; \quad y_4=\frac{1\times 10^4}{192\times 145}=0{\cdot}48;$$

$$r=\frac{y_2+y_3+y_4}{y_1+y_2+y_3}=0{\cdot}8123;$$

$$y_0=\frac{y_1+y_2+y_3}{r}-(y_1+y_2)=2{\cdot}301.$$

The population on 21 July 1949 $=\frac{10^4}{2{\cdot}301}=4345$ approximately.

It is not possible to calculate population figures for the five different dates from the data.

The estimates made from recaptures were too widely scattered to be reliable without a check, so other estimates of populations have been made from the percentage of adults which had emerged from the stumps at any one date. The assumption is made that the insects do not travel far immediately after emergence; a limited number of observations on released and recaptured marked specimens suggest this to be true.

From the data in Table 5 the mean number of adults which should have emerged from each stump, neglecting those which failed to mature in time, is approximately 35. The average stump density per plot was approximately 86 giving a total estimated emergence on the twenty-four plots of 72,240. Percentage emergence figures for dates between 19 July 1949 and 17 August 1949, calculated from Table 6, and the expected total emergence, give the population estimates shown in Table 7. The estimate from the marking experiments is given in the right-hand column for comparison.

TABLE 7. *Population estimates based on percentage emergence data*

Date	Empty pupal chambers per stump	Total live adults per stump	Emergence (%)	Estimated population	Estimated population from marking experiments
19. vii. 49	1	29·5	3·3	2,384	—
21. vii. 49	—	—	—	—	4,345
23. vii. 49	—	—	—	—	—
24. vii. 49	5	24	17·2	12,425	—
25. vii. 49	—	—	—	—	—
27. vii. 49	8	22·5	26·2	18,927	—
31. vii. 49	—	—	—	—	—
3. viii. 49	7·6	19·9	27·6	19,938	—
10. viii. 49	38	13·5	73·8	53,313	—
17. viii. 49	23	5	82·1	59,309	—

DISCUSSION

Stumps of *Pinus sylvestris* in their first year of exposure formed more important breeding sites for *Hylobius abietis* than those in their second year; the degree of infestation of first-year stumps would appear to depend upon the length of time they are exposed to oviposition. In this connexion it has been observed that the rate of egg laying is comparatively slow and the period protracted. Since the main brood of weevils does not appear until early July, and egg laying ceases in late autumn, it would seem that the old weevils, which are found from April onwards, together with those which matured too late the previous year, are quite important in building up the mass populations which follow extensive felling.

The failure to obtain reliable estimates of absolute adult populations from marking experiments may have been due to the fact that the releases of marked individuals were made at a time when the population was increasing rapidly, resulting in low recoveries, but possibly also to insufficient randomizing of the newly emerged weevils over the area as is indicated by the recapture figures in Table 6.

The writer wishes to thank Prof. J. W. Munro and Dr W. F. Jepson for their interest and encouragement. He also thanks Mr N. Fraser for assistance in the

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THE EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF THE POTATO-ROOT EELWORM, *HETERODERA ROSTOCHIENSIS*

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Experiments on the effect of temperature on the development of *Heterodera rostochiensis* are described. Temperatures above 20° C. slightly reduce penetration of larvae into the potato root and greatly restrict their development in the plant tissues. The temperature range of this parasite is compared with that of *H. marioni*, and a possible relationship to their geographical distribution is discussed.

The experiments described below were designed to investigate the reasons for failure to infect potato plants in the greenhouse with larvae of the potato-root eelworm, *Heterodera rostochiensis*.

In a preliminary experiment made in 1949 four groups, each of eight uniformly sized tubers of the variety 'Arran Banner', were planted in 6 in. pots containing a 3 : 1 mixture of cyst-free loam and sand. The four groups were maintained, respectively: (1) in a greenhouse, (2) out of doors in full sun, (3) in the shade of a north wall, (4) in pots sunk up to their necks in a gravel plunge. Each pot was infected at 2- or 3-day intervals by placing 10 c.c. of a larval suspension of newly hatched larvae of *H. rostochiensis* in the centre of each root mass. During the months of May and June ten inoculations were made, after which the potatoes were allowed to complete their growth. The soil from each pot was then dried and washed to recover any cysts. The yields of cysts from each group of eight plants were as follows: (1) in the greenhouse, 159; (2) outdoors in sunlight, 1262; (3) outdoors in shade, 2278; (4) in the plunged pots, 3215. Cysts produced under the four conditions contained an average of 33·2, 141·1, 199·8 and 295·2 larvae per cyst, equivalent to a total larval population of 5278, 178,068, 455,144 and 939,068 respectively.

As it appeared probable that the above were temperature effects, four gravel plunges were constructed to maintain temperatures of 32, 27, 21 and 15° C. respectively. Ten potato tubers (Arran Banner) in 6 in. pots of 3 : 1 cyst-free loam and sand were sunk in each plunge up to their rims. Approximately 10,000 larvae, in six doses spread over 24 days in April and May were given to each plant, which was then allowed to complete its growth. The cysts formed in each pot were extracted and their larval content estimated (Table 1). Analysis of the data shows that the differences between the numbers of cysts produced at 32, 27 and 21° C. respectively are all significant, but there is no significant difference between the figures for 21 and 15° C.; analysis of the data for larvae per cyst yields a similar result.

Thus, while the rise in temperature up to 21° C. did not check either the entry

into or the subsequent development of the larvae within the root, further rise of temperature reduced the number of new cysts and of larvae per cyst.

To differentiate between larval entry and subsequent development, eight tubers were grown at each of the four temperatures in soil infested with *H. rostochiensis* at approximately 130 larvae per g. When all the plants had sprouted they were transplanted, after washing the roots, to a 3 : 1 mixture of cyst-free loam and sand; of each set of eight plants, four were returned to their original temperature and four were placed in a single large unheated plunge. The experiment was then completed as before: and the records for each subtreatment are set out in Table 2. Analysis of the individual cyst and larval counts shows that the temperature effect on cyst number for plants maintained at their original temperature after transplanting

TABLE 1. *Total infection produced per ten plants following inoculation with newly hatched larvae of* Heterodera rostochiensis

	15° C.	21° C.	27° C.	32° C.
No. of cysts	15,603	14,434	3,581	635
Larvae per cyst	193·2	205·0	70·6	19·8
Total available larvae	3,014,499	2,958,970	252,819	12,573

TABLE 2. *Effect of transplanting infected potato plants to clean soil at the original and at cool temperatures. Data per four plants*

	Transplanting temperature	Infection temperature 15° C.	21° C.	27° C.	32° C.
Cysts per four plants	Original	11,173	4,694	1,731	141
	Cool	8,363	4,480	2,542	2,452
Larvae per four cysts	Original	771	786	404	25
	Cool	541	595	534	452
Total larvae per plant	Original	500,000	230,000	43,700	220
	Cool	282,000	167,000	80,800	69,400

showed significant increases from 32 to 27° C. and 27 to 21° C. but not from 21 to 15° C. The yield of cysts from plants transplanted to a cool plunge was not significantly different for plants infected at 32, 27 and 21° C. but showed significance when 32° C. was compared with 15° C. The effect of temperature on larval penetration, while real, was therefore small, but the larvae were unable to develop within the root at the higher temperatures. This is confirmed by the fact that significantly larger numbers of cysts were produced when plants infected at 32 and 27° C. were transplanted to cooler conditions. This significant difference is not maintained for transplants following infection at 21 and 15° C.

Similarly, when plants were maintained at their original temperatures, the differences between larvae per cyst produced at 32, 27 and 21° C. were significant, but when plants were transplanted to cool conditions temperature of infection was without effect. The difference between transplanting back to the original temperature as compared with transplanting to a cool plunge was not marked, being significant

only at 32° C. At each of the temperatures 27, 21 and 15° C. the number of larvae produced per cyst in the plants transplanted to the cool plunge did not differ significantly from the corresponding figure for the plants maintained at their original temperature.

Temperatures above 21° C. were therefore unfavourable to the development of the potato-root eelworm, and 31° C. almost inhibited development and multiplication. The temperature effect is primarily in preventing the development of the larvae within roots, although there was evidence of a small but definite effect on larval penetration.

Godfrey (1926) found that the development of *H. marioni* was negligible at 15° C.; 20° C. was required for it to become abundant; temperatures of 25–35° C. on tomatoes and 30° C. on lettuce favoured maximum development. So that, while the development of *H. marioni* is at least partially inhibited below 20° C., that of *H. rostochiensis* is checked above this temperature. *H. marioni* is a serious plant pest in U.S.A., South America and the West Indies, many regions of Africa, India, the Philippines, the East Indies, Australia, Japan, China as well as Cyprus and other Mediterranean areas. It is not usually a serious pest of field crops in temperate regions. *H. rostochiensis*, on the other hand, is widespread in north-west Europe, occurring in Great Britain, the Republic of Ireland and the Channel Islands, Sweden, Denmark, Holland, France, Belgium, Finland and some northern and north-western parts of Germany, but is not found in southern regions of Europe nor in any tropical or subtropical regions. It appears unlikely that this parasite has never been introduced into areas other than these at some time or another, and it is clear that the distribution of these two species can be broadly correlated with their temperature relationships.

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OBSERVATIONS ON THE ATTACK BY THE STEM EELWORM, *DITYLENCHUS DIPSACI*, ON STRAWBERRY

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(With Plate 16 and 1 Text-figure)

Four biologic races of *Ditylenchus dipsaci* can cause stem eelworm disease on twelve varieties of strawberry, all but one cultivated in Great Britain. The course of attack on strawberry, host reaction and the ways in which the disease originates and is disseminated are described.

Introduction

Massee (1913) was the first to report stem eelworm attack on strawberry in Britain, causing plants to rot and decay at ground-level after showing symptoms of crinkled and deformed leaves. Byars (1920) reported the disease in the United States of America from strawberry and red clover, and recorded having seen eelworm-diseased strawberry in 1915. McKay (1921) stressed the seriousness of the disease in Oregon, and the following year he found a similar disease on wild strawberry, *Fragaria chiloensis* Duchesne (McKay, 1922; Godfrey & McKay, 1924). Courtney (1936) recorded a natural transfer of infestation from red clover to strawberry, variety Marshall, in the Pacific Northwest. Later Hodson (1938), in England, reported observations on an attack by stem eelworm on strawberry varieties Madame Lefebvre and Royal Sovereign.

Experiments

In mid-August 1948 strawberry plants, variety Huxley Giant, showing crumpled growth were received from Dr Mary Noble of Edinburgh; *Ditylenchus dipsaci* had been found in these and not *Aphelenchoides fragariae* as expected (Noble, 1949). When received only very few *Ditylenchus dipsaci* were present, not enough, it seemed, to warrant the intense symptoms exhibited by the plants. In similar material sent in November, many more *D. dipsaci* were found. The plants came from a field which had grown three successive crops of field beans. *Vicia faba* is a common host of the oat race of stem eelworm, and some pots of soil were therefore sown with Golden Rain oats—a very susceptible variety—and inoculated with pieces of dried diseased strawberry plants, one pot being left uninoculated as a control. About 6 weeks later some of the oat seedlings in the inoculated pots showed slight swellings at the base and many *Ditylenchus dipsaci* were found in the tissues.

At Rothamsted there are a number of experimental plots on which different species and races of several plant parasitic nematodes are established. Among these are five carrying populations of *D. dipsaci* from oats, red clover, onion, teazle and narcissus

respectively. On to these five plots two clean plants of each of six varieties of strawberry were planted in late September 1949, and each plant was also watered with a suspension of the appropriate population of *D. dipsaci*; the varieties which had been received from East Malling Research Station were Royal Sovereign M40, Oberschlesien M42, Huxley M44, Perle de Prague M45, Tardive de Leopold F43 and Madame Lefebvre F92. By the end of March 1950 most of the plants on the oat, onion and teazle plots were showing symptoms of attack but only two varieties, Oberschlesien and Huxley, were attacked on the red clover plot, and all the plants on the narcissus plot appeared still to be clean. By June 1950 all the plants on the oat, onion and teazle plots were more or less heavily attacked. On the red clover plot all save the Royal Sovereign and one plant of Perle de Prague were attacked, and all except one Huxley and one Perle de Prague were affected on the narcissus plot. On this last plot the attack on Royal Sovereign was light and that on Madame Lefebvre were heavy. In another experiment, in which potted plants of Royal Sovereign, Huxley and Madame Lefebvre were injected by means of a hypodermic syringe* with suspensions of *D. dipsaci* from narcissus, red clover and rye, each plant showed marked symptoms after 3 or 4 months and eventually became heavily diseased.

Field observations

The strawberry variety Madame Lefebvre was severely attacked in a field at Tiptree, Essex, in 1949; these were planted in rows adjacent to the variety Huxley which showed only slight scattered infestation. The mother stock from which the Madame Lefebvre runners had been obtained was examined, but no *D. dipsaci* could be found and the origin of this Essex infestation could not be determined. In the Cheddar district in 1949 and 1950, strawberries in many fields were infested, including some in which double infestations due to parasitism by both *D. dipsaci* and *Aphelenchoides fragariae* were seen. Southey & Staniland (1950) were successful in transferring *Ditylenchus dipsaci* from oats to strawberry and from strawberry to onion, and showed that the onion race of *D. dipsaci* was probably concerned. Onions are commonly grown in the rotation, and sometimes diseased strawberry areas coincided with patches of 'bloat' in the previous onion crop. In the strawberry-growing district of Kent the variety most affected in 1949 and 1950 was Royal Sovereign, this being the most frequently grown. Infestation was also seen on Perle de Prague, Charles Lane, Madame Lefebvre, Early Cambridge and Jucunda, growing on land which had previously carried a crop of red clover, though this crop was not known to have been infested with *D. dipsaci*. Material collected from infested plants was dried slowly, and after being broken up into small pieces was used as an inoculum in pots in which Cotswold Broad red clover and Golden Rain oats were sown. Examination later showed that the red clover was infested and showed typical swellings of the leaf bases; no symptoms could be seen on the oats, though a few eelworms were recovered when the plants were extracted in a Baermann funnel.

* Seinhorst's method (personal communication).

SYMPTOMS AND METHOD OF ATTACK

Symptoms on strawberry are most marked during spring and autumn when the growth of the plant is slow compared with the reproduction of the eelworm. In summer the symptoms become largely masked by the rapid production of normal leaves which have grown too quickly for the nematode attack to become dominant.

The leaflets of infested plants are crumpled and curled backwards, the marginal teeth fewer than normal and the leaf outline, in severe attacks, very irregular. The leaf is generally more brittle, that of Huxley is slightly darker than usual; in Royal Sovereign there is a tendency to reddening, whereas in some other varieties the affected areas are often paler than normal. The lower part of the midrib is slightly wider and paler and, seen from below, slightly thickened. The affected parts of the midrib also frequently show the uneven, puffy appearance associated with attack by stem eelworm.

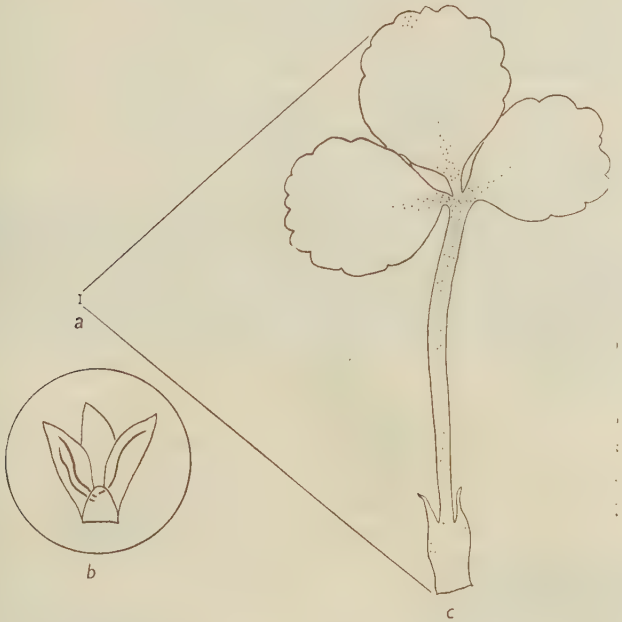
The petioles sometimes appear normal though more often they are swollen and stunted. The swellings may be general or localized, or even in the form of small, almost gall-like, enations whose epidermis is slightly rucked. The flower stalks are often much dwarfed and swollen, the swellings may be local and gall-like, and occasionally the inflorescence is almost sessile. When the plant is seriously affected the main crown may die so that the subsequent growth of lateral buds produces a multi-headed plant. The most severely attacked plants are usually killed.

These symptoms differ from those shown by plants infested by *Aphelenchoides fragariae* and *A. ritzema-bosi* where the eelworms are almost invariably ectoparasitic and the leaves often show feeding areas. These species can cause considerable irregularity of leaf outline and a loss of marginal teeth, but there is no heavy crumpling and recurving of the leaflets, nor is there any swelling of the petioles nor any swelling and widening of the midribs. The leaf does not become brittle nor does the colour change.

Ditylenchus dipsaci is endoparasitic and occurs in all parts of the plant except the roots, especially in the petiole, stipules, bases of the main leaf veins, leaf tips and within the crown. They can also be found in various parts of the inflorescence; peduncles, pedicels, sepals and in the receptacle. When ripe fruits are infested they are pale in colour and loose in texture so that they squash easily.

The tissues of the new leaf can become infested by internal migration from the crown of a badly attacked plant, though this is not the only method of attack. Dissection of parts of badly infested plants, after treatment in cotton blue-lactophenol (Franklin, 1949), shows that infestation often occurs when the leaves are very young. At this stage only the leaf base, the junction of the petiole and lamina and the leaflets are clearly defined, and they lie close together. Eelworms are found between the folds of the tiny leaflets and also partly entering the tissue at the top of the petiole. As the leaf develops, intercalary growth of the petiole separates the leaf base from the lamina and the leaf tips and petiole become separated by growth of the leaflets.

This probably accounts for the density and distribution of eelworms in a mature leaf. Most of the eelworms are found near the top of the petiole where the tissue is the first to show lesions. An infested petiole split longitudinally reveals a somewhat puffy, pale green to white dry tissue, and where the number of eelworms is greatest cells are killed and become brown. There are fewer eelworms in the lower parts of these petioles and in the proximal parts of the main veins; discontinuous patches of a few eelworms are often found in the stipules and at the leaf edges, particularly near the tips (Text-fig. 1).



Text-fig. 1. Outline diagram to show point of invasion and subsequent distribution and density of *D. dipsaci* in strawberry leaf. (*a*) Size of young leaf when invasion takes place (× 1). (*b*) Enlarged young leaf with three eelworms, their heads partly embedded in tissue at junction of leaflets and petiole initial (× 10). (*c*) Fully grown infested leaf of Huxley; dots show distribution and density of stem eelworm; note loss of marginal teeth (× $\frac{2}{3}$).

Runners from affected plants are often, though not invariably, infested. When growing from heavily attacked plants where the crown is involved the stolons are frequently stunted, thickened and distorted, and eelworms can be found throughout the tissue. Where apparently normal stolons are formed the distal daughter plants (runners) may show marked symptoms of attack. There may be occasional, isolated, dark, slightly sunken lesions along the length of the stolon from which eelworms can

usually be recovered. The runner can be infested either very early when it is a bud developing at the tip of the unextended stolon or at a later date by invasion from the soil when the runner is some distance from the parent plant.

Discussion

The earliest accounts of attack by *D. dipsaci* on strawberry in the Pacific Northwest States of U.S.A. all record red clover in association with the infestations, and describe the marked local swellings which occurred on parts of the petioles and flower stems. Similar swellings were seen on strawberry plants from Kent which were attacked by the red clover race, and also on plants grown on the red clover plot at Rothamsted. In experiments localized swellings were among the symptoms seen on plants attacked by other populations and also on plants seen in the Cheddar district. The gall-like appearance of diseased organs seems a more common feature of infestation by the red clover race than by other races of the stem eelworm.

The symptoms shown by *Fragaria chiloensis* are somewhat different; the swellings are more definitely gall-like, those on the leaves slightly resemble the 'spickles' seen on narcissus leaves. Colour photographs of infested plants collected from the Oregon coast by Dr T. Goodey in 1947 show pale green leaf galls, occasionally somewhat reddened, on dark green leaves. There is some deformation but no real crinkling of the leaves. Further work is needed on the pathology of stem eelworm attack on *F. chiloensis*.

In the experiments described populations from six different host plants—oats, onion, red clover, teazle, narcissus and rye—were tested and all were able to attack strawberry and cause disease. There is clear evidence for the identity of the oat-onion race, and recent observations by T. Goodey (personal communication) indicate that rye is another host of this race. Thus only four biologic races, namely, oat-onion-rye, red clover, narcissus and teazle, occur among the six populations of stem eelworm tested.

Records and observations show that the varieties of strawberry susceptible to attack include Marshall, Sir Joseph Paxton (Noble, 1949), Huxley, Royal Sovereign, Madame Lefebvre, Perle de Prague, Oberschlesien, Tardive de Leopold, Climax (Pitcher, in litt.), Jucunda, Charles Lane and Early Cambridge. The experiments did not indicate differences in varietal susceptibility, though from the observations made at Tiptree and by Dr Pitcher at East Malling it is tentatively suggested that Huxley may be less susceptible than other varieties.

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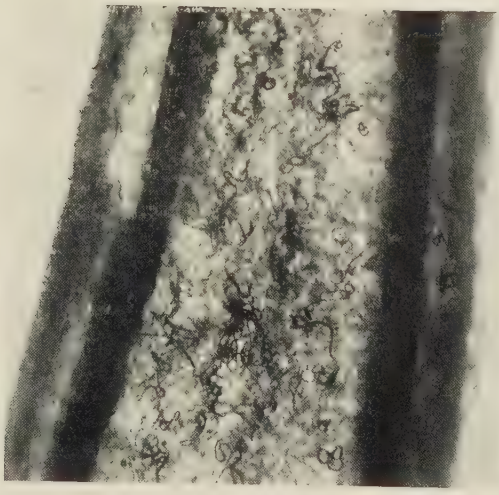
A

B



C

GOODEY—*Stem eelworm* Ditylenchus dipsaci *on strawberry*

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EXPLANATION OF PLATE 16

A. Strawberry, Perle de Prague, attacked by red clover race of *D. dipsaci*. Note swellings on petioles and peduncles.

B. Strawberry, Huxley Giant, infested with oat race of *D. dipsaci*, showing crinkling of leaves and short internodes of stolons.

C. Longitudinal section of strawberry petiole, var. Madame Lefebvre; eelworms and eggs stained with osmic acid. (Eelworms about 1 mm. long.)

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SOME INTERRELATIONSHIPS BETWEEN MANGANESE, MOLYBDENUM AND VANADIUM IN THE NUTRITION OF SOYBEAN, FLAX AND OATS

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(With Plates 17 and 18)

Soybean and flax were grown in nutrient solutions containing high and low levels of molybdenum and vanadium, in combination with toxic (10–25 p.p.m.) and non-toxic (1 p.p.m.) manganese. Molybdenum (20 and to a less extent 10 p.p.m.) intensified the chlorosis induced by manganese excess, though these concentrations were harmless in the presence of 1 p.p.m. Mn. Vanadium (≡ 1·0, 5 and 10 p.p.m. Mo) counteracted some of the symptoms of manganese toxicity, but the two higher rates were harmful to growth irrespective of the manganese supply. Toxic concentrations of vanadium at first deepened the green colour of the shoot, though apical iron-deficiency chlorosis was generally induced later. Low molybdenum (0·1 p.p.m.) or equivalent vanadium had no influence on growth or iron nutrition at either level of manganese. Visual differences were corroborated by changes in the nitrogen, phosphorus and iron contents of the plants. There was no evidence of replaceability of molybdenum by vanadium.

Oats were grown in nutrient solutions containing various combinations of manganese (nil–400 p.p.m.) and molybdenum (nil–20 p.p.m.). The appearance of manganese-deficiency symptoms was not affected by the quantity of molybdenum provided, and the manganese and molybdenum contents of the leaves were mutually independent of the quantity of each element supplied.

I. Introduction

Recent advances in knowledge regarding the effect of trace elements on plant growth show that the question is more complex than was originally thought, and that interrelationships between the various major and minor elements are of paramount importance in maintaining the plant in a healthy condition. Liebig, Vanselow & Chapman (1942) indeed state 'the antagonistic effect of a trace of one element upon another may profoundly influence plant growth and yet mean nothing with regard to essentiality'. Millikan has devoted considerable attention to such interactions, and working with flax found (1947) that molybdenum and (1948) that aluminium could counteract the iron deficiency induced by excess manganese and some other elements in nutrient solutions. From the resemblance between the symptoms of molybdenum deficiency and manganese toxicity (1948), and from pot and field experiments (1949*a*), he concluded that the two elements were antagonistic. Iron nutrition is affected by excess of heavy metals, and Millikan (1947) attributed the beneficial action of molybdenum in overcoming the toxic effect of manganese to its

being 'associated with the regulation of the...physiological availability of iron to the plant'. Hewitt (1949) compared the ability of a large number of metals to induce iron deficiency. Though his results on sugar beet regarding the interaction of molybdenum with manganese did not agree with those of Millikan on flax, he suggested that some aspect of nitrogen nutrition is nevertheless probably involved in either case. Bennett (1945) has put forward the view that 'chlorosis is a disturbance of nitrogen metabolism as well as of iron metabolism', and the fact that manganese and molybdenum play a part in nitrate reduction fits in with such a fourfold relationship.

The main object of the present investigations was to make a further study of this manganese-molybdenum relationship on crops such as flax, soybean and oats, which were likely to show differences in their requirements for the two elements. The plants were grown in nutrient solutions containing various combinations of manganese and molybdenum at levels known to be adequate, and also at concentrations expected to be toxic. In the experiments on oats, no-manganese and no-molybdenum treatments were also included. At the suggestion of Dr P. J. G. Mann, the effect of vanadium was compared with that of equivalent molybdenum on flax and soybean, for it seemed possible that the two elements might react similarly towards manganese. Horner, Burk, Allison & Sherman (1942) obtained evidence of the replaceability of molybdenum by vanadium in the nutrition of azotobacter, though Vanselow & Datta (1949) found that this did not hold for citrus.

II. Experimental procedure

Crone nutrient solution (pH 6·4) was used for soybean and flax, but oats were grown in a Rothamsted solution with phosphates adjusted to give a pH of 4·7, and ferric citrate as the source of iron (Warington, 1950). The seeds, graded by weight for soybean and oats, were germinated on waxed netting stretched over beakers containing the appropriate basal solution diluted to $\frac{1}{5}$. No manganese or molybdenum was supplied at this stage, though boron was included. The plants were then grown singly in bottles of 400 or 600 c.c. capacity, flax having a preliminary period of about 2 weeks in 100 c.c. vessels. Solutions were renewed fortnightly. Five replicates were set up for each treatment and were bulked for chemical analysis. Manganese was supplied as $MnSO_4.4H_2O$, molybdenum as $Na_2MoO_4.2H_2O$, and vanadium as $VOCl_2$. Since the molecular weight of molybdenum is nearly double that of vanadium, the actual concentration of vanadium used was very much lower than the molybdenum.

Total-nitrogen was estimated by the micro-Kjeldahl method and nitrate-nitrogen by reduction with iron (Humphries, 1951). Phosphorus and iron were determined colorimetrically, the former by means of stannous chloride reduction, and the latter by the thiocyanate method. It was realized that the Crone nutrient solution was not ideal for a study of the uptake of phosphate or iron, but useful preliminary information was nevertheless obtained.

A few painting tests were carried out with various solutions on flax, to determine the cause of apical chlorosis, 0·05% sulphonated lorol being used as spreader.

In view of the great variability in size of the plants within each experiment, analyses of variance of yield were worked out after conversion to logarithms. No standard error appropriate to the data in the tables is, therefore, available.

III. SOYBEAN

Two experiments were carried out in 1950 with soybean variety Fiskeby II. No inoculations with *Rhizobium* were made and the plants remained free of nodules. Treatments were as follows:

Exp. I (3 May–28 June). 1·0 and 25 p.p.m. Mn, each combined with 0·1 or 10 p.p.m. Mo or equivalent V.

Exp. II (29 June–5 September). 1·0, 10 and 20 p.p.m. Mn, each combined with 0·1, 10 or 20 p.p.m. Mo, or V equivalent to 0·1, 1·0 or 10 p.p.m. Mo, the smaller range being chosen for vanadium owing to its toxicity.

Treatments were started 6–7 days after sowing. The results from both experiments will be considered together on the basis of the level of manganese provided.

(1) *Manganese*, 1 *p.p.m.*

This concentration of manganese served as the control, since it was known to maintain healthy growth of soybean without producing either deficiency or toxicity symptoms when solutions were renewed fortnightly.

Molybdenum, 0·1, 10 and 20 p.p.m. Throughout the whole growing period there was no apparent difference between the plants supplied with any of these levels of molybdenum, and their final yields were very similar (Tables 1 and 2). The absence of any sign of toxicity with the highest concentration was specially noteworthy (Pl. 17, fig. 1). Flowering and pod formation was normal, and the leaves were healthy, though the lower ones became yellowed and speckled as they matured, due probably to normal senescence. The roots were well developed, long and fibrous.

Vanadium (≡0·1, 1·0 and 10 p.p.m. Mo). When vanadium was supplied at the two lowest levels (≡0·1 or 1·0 p.p.m. Mo), the plants closely resembled those grown with molybdenum both in appearance and yield. The highest concentration (≡10 p.p.m. Mo), however, was very toxic (Pl. 17, fig. 2), injury being well marked after 2 weeks' growth. The primary roots were dwarfed, swollen and gelatinous, often splitting in the upper regions revealing a mass of corky tissue, while lateral growth was almost entirely inhibited (Pl. 17, fig. 4, left). The shoots were healthy at first, and of a dark blue-green colour similar to that described by Scharrer & Schropp (1935) for other vanadium-treated plants. As they found also, apical chlorosis developed later, though its extent varied considerably between the two experiments, probably on account of environmental differences. Evidence regarding the effect of season is, however, conflicting, for Hopkins, Págan & Ramírez Silva

TABLE 1. *Yield and nitrogen content of soybeans grown with different combinations of manganese, molybdenum and vanadium. (Mean of 5 plants.) Exp. I*

Treatment	Dry weight (g.)			Total-N (% d.m.)		NO_3-N (% d.m.)	
	Shoot	Root	Total	Shoot	Root	Shoot	Root
			(Mn, 1 p.p.m.)				
Mo, 0·1 p.p.m.	3·74	0·96	4·70	2·09	2·27	0·02	0·03
Mo, 10·0 p.p.m.	4·32	1·02	5·34	1·89	2·09	0·02	0·05
V≡0·1 p.p.m. Mo	4·40	0·98	5·38	1·87	1·97	0·02	0·01
V≡10·0 p.p.m. Mo	1·05	0·28	1·33	1·70	2·42	0·03	0·11
			(Mn, 25 p.p.m.)				
Mo, 0·1 p.p.m.	0·68	0·22	0·90	3·66	3·76	0·08	0·55
Mo, 10·0 p.p.m.	0·41	0·16	0·57	4·24	4·16	0·14	0·47
V≡0·1 p.p.m. Mo	0·80	0·28	1·08	3·70	4·01	0·08	0·61
V≡10·0 p.p.m. Mo	0·60	0·21	0·81	2·33	2·30	0·03	0·08

TABLE 2. *Yield and composition of soybeans grown with different combinations of manganese, molybdenum and vanadium. (Mean of 5 plants.) Exp. II*

Treatment	Dry weight (g.)			Total-N (% d.m.)		NO_3-N (% d.m.)		P (% d.m.)	Fe (% d.m.)
	Shoot	Root	Total	Shoot	Root	Shoot	Root	Shoot	Shoot
				(Mn, 1 p.p.m.)					
Mo, 0·1 p.p.m.	9·57	1·88	11·45	1·58	1·75	0·01	0·01	0·28	0·043
Mo, 10·0 p.p.m.	10·97	2·22	13·19	1·38	1·51	0·01	0·01	0·22	0·035
Mo, 20·0 p.p.m.	10·99	2·18	13·17	1·54	1·54	0·01	0·01	0·22	0·049
V≡0·1 p.p.m. Mo	10·99	2·37	13·36	1·39	1·36	0·01	0·01	0·21	0·040
V≡1·0 p.p.m. Mo	11·07	2·40	13·47	1·43	1·30	0·01	0·01	0·20	0·041
V≡10·0 p.p.m. Mo	0·81	0·38	1·19	2·14	1·84	0·01	0·02	0·23	0·009
				(Mn, 10 p.p.m.)					
Mo, 0·1 p.p.m.	3·63	0·63	4·26	2·17	2·86	0·01	0·23	0·32	0·021
Mo, 10·0 p.p.m.	3·30	0·67	3·97	2·36	3·30	0·01	0·31	0·34	0·018
Mo, 20·0 p.p.m.	0·56	0·18	0·74	4·30	2·79	0·12	0·29	0·28	0·010
V≡0·1 p.p.m. Mo	2·08	0·61	2·69	3·03	3·72	0·01	0·51	0·48	0·010
V≡1·0 p.p.m. Mo	3·89	0·93	4·82	1·94	1·95	0·01	0·01	0·45	0·015
V≡10·0 p.p.m. Mo	0·46	0·18	0·64	2·61	2·89	0·01	0·04	0·67	0·013
				(Mn, 20 p.p.m.)					
Mo, 0·1 p.p.m.	1·29	0·39	1·68	3·04	3·79	0·01	0·34	0·31	0·008
Mo, 10·0 p.p.m.	0·38	0·17	0·55	4·08	3·26	0·16	0·19	0·51	0·009
Mo, 20·0 p.p.m.	0·37	0·19	0·56	4·53	3·33	0·20	0·23	0·59	0·006
V≡0·1 p.p.m. Mo	1·26	0·34	1·60	3·34	4·22	0·01	0·34	0·69	0·007
V≡1·0 p.p.m. Mo	1·87	0·51	2·38	3·08	3·41	0·01	0·43	0·51	0·007
V≡10·0 p.p.m. Mo	0·54	0·27	0·81	3·04	2·32	0·08	0·05	0·56	0·014

(1944) found that high light intensity encouraged chlorosis in pineapple, whereas Gile (1916) and several others have reported increased chlorosis during the winter months. According to Chapman (1931) and Jones (1938) temperature may also influence its development. In the first experiment with soybean, the apical chlorosis which developed in two of the five replicates only slightly reduced the dry weights

of these shoots, and was without influence on the yield of roots. It seemed unlikely, therefore, that its incidence was due to a simple lack of iron through root restriction. An accumulation of ferrous phosphate on the surface of the roots supported this view. In the second set, the chlorosis was more severe and appeared in four of the replicates, and though the lower leaves retained their green colour they became slightly speckled brown. From analogy with flax which responded to painting with ferrous sulphate when treated with similar quantities of manganese and vanadium, there seems every reason to attribute the apical chlorosis in soybean to vanadium-induced iron deficiency, as Hewitt (1949) has described for sugar beet. Chapman (1931), on the other hand, working with injections on fruit trees, found that vanadium had no effect on chlorosis, though the element was very toxic.

(2) *Manganese*, 10, 20 *and* 25 *p.p.m.*

Plants supplied with low molybdenum combined with high manganese served as the standard for manganese excess symptoms, since with the same concentration of molybdenum in the presence of only 1 p.p.m. Mn, growth was perfectly healthy. The symptoms for soybean confirmed those illustrated in *Hunger Signs in Crops* (1941) and were briefly as follows. The plants were stunted, generally failing to twine, and the root system, though normal in type, was poor and discoloured brown. Puckering of the leaves, particularly in the upper part of the shoot, also noted by Morris & Pierre (1949), was very characteristic, and apical chlorosis occurred when the symptoms were severe (Pl. 17, fig. 3, right). The lower leaves usually remained green but always had brown spots.

As would be expected from Millikan's results (1947) with flax, the effect of adding molybdenum or vanadium to nutrient solutions containing excess manganese depended on the relative concentrations of each pair of elements. The results with manganese at 20 or 25 p.p.m. were so similar that they will be taken together, but those at the 10 p.p.m. level showed certain important differences and will be considered separately.

(*a*) 10 *p.p.m.* Mn

Molybdenum, 0·1, 10 *and* 20 *p.p.m.* With 0·1 or 10 p.p.m. Mo the symptoms of manganese excess were well defined but not severe. The upper leaves were crinkled and the lower ones spotted but no apical chlorosis developed until just before harvest. The dry weights (compared with similar molybdenum treatments at the 1 p.p.m. Mn level) were significantly reduced in each case (Table 2). In the presence of 20 p.p.m. Mo, manganese toxicity symptoms were intensified and apical chlorosis was considerable (Pl. 18, fig. 5). This could not have been due to molybdenum poisoning alone, since plants had tolerated a similar concentration in combination with 1 p.p.m. Mn (Pl. 17, fig. 1, right), and, further, there was no sign of the golden yellow colour usually associated with molybdenum excess (Warington, 1937; Millikan, 1949*b*). This increase in manganese toxicity from the addition of high

molybdenum was borne out by a further and significant reduction in dry weight (Table 2), confirming the findings of Hewitt (1949) rather than those of Millikan (1947). It should, however, be noted that Millikan found the effectiveness of molybdenum in counteracting manganese excess was greatest within a range of 5–25 p.p.m. Mo, quantities which, according to his later work, were liable to be toxic (1949*b*). The fact that no injury was experienced with the rate of molybdenum used in the present experiments (20 p.p.m.) may possibly explain the difference in results.

Vanadium (≡0·1, 1·0 *and* 10 *p.p.m.* Mo). When supplied with vanadium equivalent to 0·1 or 1·0 p.p.m. Mo, the symptoms of manganese toxicity were at first very similar in type and intensity to those described for the two lowest molybdenum treatments, but after the first 3 weeks there was noticeably less leaf crinkling where the larger quantity of vanadium had been given (Pl. 18, fig. 6). This improvement was confirmed by an increase in the dry weight produced (Table 2). With vanadium at the highest rate (≡10 p.p.m. Mo), the decrease in leaf crinkling was even more striking, while the lower leaf spotting was also much reduced. Apical chlorosis, on the other hand, was accentuated both on the main and axillary shoots, and the final yield was very small (Pl. 17, fig. 4, centre). Thus it seemed that vanadium, when present in sufficient quantity, could to some extent counteract the harmful effect of excess manganese, as Millikan (1949) has described for molybdenum and aluminium in the case of flax. The aggravation of the apical chlorosis with the highest rate of vanadium appears at first sight conflicting. At this concentration, however, vanadium is injurious on its own account, some apical chlorosis and abnormal root growth appearing in the presence of a non-toxic (1 p.p.m.) level of manganese (Pl. 17, fig. 4, left). That the plants were suffering from vanadium excess was evident from their roots, and it is probable that the chlorosis was the result of a superimposed vanadium toxicity and not due to any accentuation of the manganese excess as was the case with high molybdenum.

(*b*) 20 *or* 25 *p.p.m.* Mn

No direct comparison can be made between the effects of these two concentrations of manganese, since the plants receiving them were not grown under identical conditions, but both produced severe toxicity symptoms as already described, including apical chlorosis.

Molybdenum, 0·1, 10 *and* 20 *p.p.m.* In the presence of either 0·1 or 10 p.p.m. Mo, 25 p.p.m. Mn produced signs of manganese toxicity after about 2 weeks. The larger quantity of molybdenum hastened the development of the symptoms and significantly reduced the dry weight (Table 1), results supported in the later experiment where the apical chlorosis was intensified and the yield reduced by both 10 and 20 p.p.m. Mo when combined with 20 p.p.m. Mn (Pl. 18, fig. 7 and Table 2). The two elements, therefore, seemed to be working in an additive rather than in an antagonistic manner, for whereas in the 10 p.p.m. Mn series, 20 p.p.m. Mo were

required to induce severe chlorosis, similar injury was produced with only 10 p.p.m. Mo if the manganese was doubled (cf. Pl. 18, figs. 5, 7).

Vanadium (≡0·1, 1·0 *and* 10 p.p.m. Mo). With 25 p.p.m. Mn, plants receiving vanadium ≡0·1 p.p.m. Mo showed signs of manganese toxicity after about 2 weeks, and as regards leaf symptoms, apical chlorosis and dry weight closely resembled those receiving equivalent molybdenum. With an increase in vanadium (≡10 p.p.m. Mo), the resemblance ceased, the colour of the leaves deepened, spotting was only slight and practically no crinkling developed at all. Apical chlorosis, however, continued and the roots showed the malformation characteristic of vanadium poisoning, but the difference in yield between the plants at the two levels was not significant in contrast to the results with molybdenum (Table 1). In the second experiment, where 20 p.p.m. Mn was given, the effects of 0·1 p.p.m. Mo or equivalent V on appearance and yield were again almost identical, symptoms of manganese toxicity being well defined (cf. plants on left, Pl. 18, figs. 7, 8).

An increase in vanadium (≡1·0 p.p.m. Mo) brought about a slight improvement in colour, but there was no marked benefit as had been the case when the same level of vanadium was combined with 10 p.p.m. Mn, and the rise in yield barely reached significance (Table 2 and Pl. 18, fig. 8). The striking visual effects of a further addition of vanadium (≡10 p.p.m. Mo) obtained in the first experiment, however, were fully confirmed, leaf crinkling being almost entirely eliminated, though apical chlorosis was severe and the youngest leaves withered (Pl. 17, fig. 4, right). The roots showed the characteristic injury and there was a significant decrease in yield (Table 2).

(3) *Nitrogen content*

Both manganese (Burström, 1939), and molybdenum (Mulder, 1948), are regarded as essential for nitrate reduction and, in consequence, a deficiency of either element is usually associated with an increase in percentage total-nitrogen and particularly of nitrate-nitrogen in the plants (Leeper, 1941; Hewitt & Jones, 1947). Although in the experiments now under discussion, there was no question of the plants lacking manganese or molybdenum, it was possible that differences in their nitrogen contents might throw some light on the interaction, if any, between manganese, molybdenum and vanadium. A rise in percentage nitrogen, however, is a common occurrence when nutritional conditions are adverse, a fact borne out in the present investigation.

Total nitrogen (*percentage dry matter*)

Excess manganese (10 p.p.m. or more) caused a definite increase in percentage total nitrogen in the shoot, the highest values being reached where additional molybdenum had intensified its toxic action (Tables 1 and 2). A similar increase occurred in the roots, but here the association with molybdenum was lacking. Injury from excess vanadium (≡10 p.p.m. Mo) in the presence of low manganese (1 p.p.m.) was also reflected in Exp. II by a rise in nitrogen content of the shoot. On the other

hand, a fall rather than a rise generally occurred in both shoot and root when excess vanadium was combined with excess manganese, compared with low vanadium under the same conditions, suggesting an interaction between the two elements. This effect was particularly noticeable with V ≡ 10 p.p.m. Mo combined with 25 p.p.m. Mn, and V ≡ 1·0 p.p.m. Mo with 10 p.p.m. Mn, where such an interrelationship had been indicated by visual differences. Since improvement in colour was a feature of these plants, it seems likely that increased carbohydrate production and hence better utilization of the nitrogen absorbed at least partly accounted for the fall in nitrogen content. Similarly, the chlorosis induced by excess manganese or manganese plus molybdenum, with resulting reduction in carbohydrate formation, may to some extent explain the rise in nitrogen content that occurred under these conditions.

Nitrate-nitrogen (*percentage dry matter*)

The influence of the various molybdenum-manganese treatments on the percentage nitrate-nitrogen content was most clearly defined in the shoot in spite of the low values obtained. Provided manganese was supplied at the non-toxic rate of 1 p.p.m., the nitrate content was negligible, and neither raising the manganese level alone, nor the addition of excess vanadium brought about any increase, as had been the case with total nitrogen. A definite rise, however, occurred with those combinations of excess manganese and high molybdenum that had encouraged chlorosis and increased the total-nitrogen content of the shoot (Tables 1 and 2). In the root, where the values for nitrate-nitrogen were considerably higher, this effect of excess molybdenum in the presence of excess manganese did not appear. High vanadium, combined with high manganese, on the other hand, appreciably decreased the nitrate-nitrogen content, again suggesting some counter-effect of vanadium on manganese toxicity. In general, the influence of the various treatments on growth was reflected in the percentage of both total and nitrate nitrogen in the plants, the contrasting action of high molybdenum and high vanadium in the presence of excess manganese being particularly well brought out.

(4) *Phosphorus and iron contents*

Relationships between phosphorus and iron in plant nutrition are well recognized, and it was possible that information regarding their uptake might throw light on the interpretation of metal-induced chlorosis. Material was available from one experiment only (Table 2). At the lowest level of manganese, the percentage phosphorus content in the dry matter of the shoot was unaffected by the amount of molybdenum or vanadium provided. With an increase in manganese to 10 or 20 p.p.m., a rise in phosphorus occurred with all levels of vanadium, but with molybdenum only at the two higher rates in the presence of 20 p.p.m. Mn. Differences in iron content were better defined, a marked reduction occurring in all chlorotic plants whether this condition was induced by manganese or vanadium. Indication of a corrective action of high vanadium on excess manganese was obtained in one instance.

IV. Flax

Three experiments were carried out with flax in 1950, the variety Liral Prince being grown in each case. Treatments were as follows:

Exps. III and IV (28$\frac{1}{4}$March–19 June). 1·0 and 25 p.p.m. Mn, each combined with 0·1 and 10 p.p.m. Mo or equivalent V, the 10 p.p.m. Mo rate being raised to 20 p.p.m. after 1 month in Exp. III. Many plants in Exp. III suffered from a slight temporary iron-deficiency chlorosis during the first 2 weeks of growth. Since apical chlorosis was one of the effects looked for, all treatments were repeated (as Exp. IV) on older seedlings, which had retained their green colour from the start. They originated from the same sowing as Exp. III, but had been kept 11 days longer in the dilute basal nutrient solution before treatments were started.* The results from the two series, however, were in complete agreement on all important points.

Exp. V (2 August–20 September). 1.0 and 20 p.p.m. Mn, each combined with 0·1, 5·0 and 10 p.p.m. Mo or equivalent V.

Treatments were started 9, 20 and 8 days from sowing in Exps. III–V respectively. The results will be discussed together and grouped on the basis of the manganese level provided.

TABLE 3. *Yield and phosphorus content of flax grown with different combinations of manganese, molybdenum and vanadium.* (*Mean of 5 plants.*) *Exp. III*

	Mn, 1 p.p.m.				Mn, 25 p.p.m.			
	Dry weight (g.)			P (% d.m.)	Dry weight (g.)			P (% d.m.)
Treatment	Shoot	Root	Total	Shoot	Shoot	Root	Total	Shoot
Mo, 0·1 p.p.m.	5·72	0·57	6·29	0·28	0·47	0·12	0·59	0·49
Mo, 10–20 p.p.m.	5·60	0·54	6·14	0·35	0·07	0·04	0·10	0·64
V≡0·1 p.p.m. Mo	4·56	0·50	5·06	0·30	0·51	0·13	0·64	0·47

(1) *Manganese, 1 p.p.m.*

Molybdenum, 0·1, 5·0 and 10–20 p.p.m. The plants receiving 0·1 p.p.m. Mo in the presence of 1 p.p.m. Mn served as control. Flax proved tolerant to molybdenum, and in the spring experiments showed no toxic effects either as regards yield or appearance with a concentration of 10 p.p.m. Mo, even if raised to 20 p.p.m. after 1 month (Tables 3 and 4 and Pl. 18, figs. 9, 10). Some chlorosis, however, developed in the autumn-grown plants with 10 and to a less extent with 5 p.p.m. Mo, though it did not reduce their yield (Table 5). Millikan (1949*b*) has reported damage to flax seedlings at even lower levels. A slightly higher dry weight was obtained in one instance with 10 compared with 0·1 p.p.m. Mo (Table 4), but in general, as for soybean, the ultimate effect was similar with all the concentrations of molybdenum tried, provided manganese was at the 1 p.p.m. level.

* Temporary iron chlorosis in seedlings grown in nutrient solution is common and usually attributed to inaccessibility of the iron while the roots are short. This result suggests that transfer to a more concentrated nutrient medium may increase the demand for iron.

Vanadium (≡0·1, 5 *and* 10 *p.p.m.* Mo). When supplied at the lowest rate, the effect of vanadium closely resembled that of molybdenum, both as regards appearance and yield, the increase in dry weight with vanadium in Exp. IV not being quite significant (Table 4 and Pl. 18, figs 9, 10). With a rise in the concentration of vanadium (≡5 or 10 p.p.m. Mo) the similarity ceased, both levels proving highly toxic to

TABLE 4. *Yield, phosphorus and iron contents of flax grown with different combinations of manganese, molybdenum and vanadium.* (*Mean of 5 plants.*) *Exp. IV*

Treatment	Height (cm.) Shoot	Dry weight (g.) Shoot	Root	Total	P (% d.m.) Shoot	Fe (% d.m.) Shoot
		(Mn, 1 p.p.m.)				
Mo, 0·1 p.p.m.	118	2·38	0·29	2·67	0·27	0·002
Mo, 10·0 p.p.m.	122	3·38	0·49	3·87	0·28	0·001
V≡0·1 p.p.m. Mo	124	3·16	0·37	3·53	0·27	0·002
V≡10·0 p.p.m. Mo	36	0·11	0·02	0·13	0·11	0·038
		(Mn, 25 p.p.m.)				
Mo, 0·1 p.p.m.	73	0·97	0·18	1·15	0·31	0·004
Mo, 10·0 p.p.m.	62	0·72	0·16	0·88	0·40	0·004
V≡0·1 p.p.m. Mo	80	1·10	0·24	1·34	0·30	0·004
V≡10·0 p.p.m. Mo	36	0·11	0·02	0·13	0·09	0·049

TABLE 5. *Effect of different combinations of manganese, molybdenum and vanadium on growth of flax.* (*Mean of 5 plants.*) *Exp. V.*

Treatment	Height (cm.) Shoot	Dry weight (g.) Shoot	Root	Total
	(Mn, 1 p.p.m.)			
Mo, 0·1 p.p.m.	58·8	0·75	0·21	0·96
Mo, 5·0 p.p.m.	59·0	0·81	0·26	1·07
Mo, 10·0 p.p.m.	50·0	0·57	0·17	0·74
V≡0·1 p.p.m. Mo	58·8	0·79	0·23	1·02
V≡5·0 p.p.m. Mo	11·4	0·03	0·02	0·05
V≡10·0 p.p.m. Mo	12·8	0·03	0·02	0·05
	(Mn, 20 p.p.m.)			
Mo, 0·1 p.p.m.	10·6	0·06	0·02	0·08
Mo, 5·0 p.p.m.	15·0	0·05	0·03	0·08
Mo, 10·0 p.p.m.	15·0	0·05	0·03	0·08
V≡0·1 p.p.m. Mo	20·8	0·09	0·04	0·13
V≡5·0 p.p.m. Mo	12·8	0·03	0·01	0·04
V≡10·0 p.p.m. Mo	14·4	0·04	0·02	0·06

shoot and root (Tables 4 and 5). The symptoms of poisoning appeared in the root after 10–14 days' treatment, but differences in shoot colour developed within a week and closely resembled those described for soybean. In Exp. III, vanadium equivalent to 10 p.p.m. Mo prevented the early chlorosis that occurred with all other treatments, and in Exp. IV (where no actual chlorosis developed) turned the shoots a deep blue-green colour. In the fifth experiment, adpression of the leaves in an

upward direction was an additional symptom. An example of such differences in colour intensity is given in Table 6. The estimations were made after 10 days' treatment, when all but the 10 p.p.m. Mo set (1 p.p.m. Mn series) had recovered from their initial chlorosis and when toxicity from 20 p.p.m. Mn had set in. The colour was assessed numerically by eye and checked on Klingksieck and Valette's *Code des Couleurs*. As with soybean, this intensification of colour occurred in plants with a restricted root growth, which might have been expected to induce rather than prevent iron-deficiency chlorosis. The subsequent colour developments with high vanadium varied. In Exp. III severe apical chlorosis rapidly set in. A response to painting with ferrous sulphate (see below) showed that this was due to iron deficiency, but the plants eventually died. On the other hand, no chlorosis developed in Exp. IV,* though both shoots and root growth were much reduced. In Exp. V, where two levels of vanadium, both toxic, were tried, the result depended on the amount of the element provided. Chlorosis followed rapidly in the presence of vanadium ≡ 5 p.p.m. Mo, but was both less severe and slower to develop if the concentration was doubled. It would seem, therefore, that vanadium could influence the iron nutrition of flax in much the same way as had been found for soybean. For a limited time and within a limited range of concentrations it could prevent iron deficiency, and yet, under other conditions, itself induce symptoms of iron chlorosis even when there was no question of accompanying manganese excess.

(2) *Manganese, 20 and 25 p.p.m.*

Since the plants grown with 0·1 and 1 p.p.m. Mo were healthy, any difference between them and those supplied with a similar concentration of molybdenum combined with high manganese may fairly be ascribed to the increase in the latter element. On this basis, both 20 and 25 p.p.m. Mn proved very toxic, symptoms appearing 6–12 days after treatment was started. The chief visual effects in the shoot were reduction in height, apical chlorosis and brown speckling on the lower leaves as described by Millikan (1949*b*). The roots were normal in type, but poorly developed and usually discoloured. Dry weights of both shoot and root were significantly reduced (Tables 3–5).

Molybdenum, 0·1, 5 and 10–20 p.p.m. In the presence of 20 or 25 p.p.m. Mn, all plants receiving 0·1–10 p.p.m. Mo showed symptoms of manganese excess to much the same extent, though there was some evidence from accentuated chlorosis that the 10 p.p.m. Mo rate was beginning to enhance the harmful effect of 25 p.p.m. Mn. The reduction in dry weight did not reach significance in this case, but at least there was no rise in yield as had followed a similar increase in molybdenum at the lower level of manganese (Table 4). Raising the molybdenum from 10 to 20 p.p.m. after 1 month's growth, on the other hand, clearly aggravated the toxicity of the manganese, the reduction in dry weight proving significant (Table 3 and Pl. 18, fig. 9). Ultimately these plants became a golden yellow colour suggestive of molybdenum poisoning,

* The roots had a better start here than in Exp. III and this may have been a causative factor.

though no such symptom had appeared in plants receiving the same quantity of molybdenum combined with low (1 p.p.m.) manganese. Thus within the limits of these experiments, high molybdenum intensified rather than counteracted the effects of manganese excess on flax, a result which confirmed that obtained with soybean.

Vanadium (≡0·1, 5 *and* 10 *p.p.m.* Mo). Plants grown with the lowest concentration of vanadium closely resembled those with equivalent molybdenum at the corresponding levels of manganese, both as regards the degree of apical chlorosis, lower leaf spotting and final dry weights (Tables 3 and 4; Pl. 18, figs. 9, 10). At higher rates (≡5 or 10 p.p.m. Mo), the two elements had quite different effects, vanadium proving very toxic. In spite of their reduction in size, the colour of the shoots was noticeably deepened, and in Exp. III the highest level of vanadium entirely suppressed the early apical chlorosis. As had been the case in the low

TABLE 6. *Colour scores of shoot apices. Flax.* (*Mean of 5 plants.*) *Exp. V*

Treatment (p.p.m.)	Mn, 1 p.p.m.	Mn, 20 p.p.m.
Mo, 0·1	6·4	4·0
Mo, 5·0	6·6	4·0
Mo, 10·0	5·4	4·2
V≡Mo, 0·1	6·8	4·4
V≡Mo, 5·0	6·8	6·2
V≡Mo, 10·0	10·0	9·6

Deep green, 10 Normal green, 6–7 Chlorotic, <6

manganese group, however, these plants eventually died in a chlorotic condition, due presumably to vanadium poisoning. Similar treatment prevented both apical chlorosis and lower leaf spotting in the second experiment, and considerably reduced their severity in Exp. V (Table 6). With half the quantity of vanadium, the symptoms were merely delayed. These visual effects confirm those obtained with soybean, but the concentrations used for flax were evidently too high for any beneficial effect to be reflected in the dry weight (Table 5).

(3) *Painting tests*

Duplicate spare plants from Exps. III and IV, all showing chlorosis, were painted at their apices with solutions of iron, molybdenum or vanadium, and the result compared with untreated controls. The chief points established were:

(1) 0·25% ferrous sulphate restored the colour of plants grown with (*a*) high manganese, in conjunction with non-toxic (0·1 p.p.m.) molybdenum or equivalent vanadium, (*b*) high vanadium, in conjunction with toxic or non-toxic manganese.

(2) Solutions of 10 or 20 p.p.m. Mo or equivalent V were ineffective.

That iron deficiency was invariably the cause of chlorosis, regardless of how it had been induced, was confirmed by the recovery of all plants in Exp. V after painting with ferrous sulphate. Spotting on the lower leaves in the presence of excess manganese, however, persisted after the recovery at the apex, in accordance with Millikan's

results (1949*b*), and supports Hewitt (1946) and others in refuting the claim of Somers & Shive (1942) that iron-deficiency symptoms are identical with those of manganese excess.

(4) *Phosphorus and iron contents*

The dark blue-green colour and adpression of the leaves in flax receiving high vanadium was reminiscent of both phosphorus starvation and excess iron conditions, a feature also noted by Millikan (1949*b*). Shoots of plants which had shown leaf colours ranging from chlorotic to deep green were accordingly analysed for phosphorus and iron (Tables 3 and 4). In general, when manganese was supplied at a toxic level, the percentage phosphorus content in the dry matter was increased as Millikan (1949*a*) found, a further rise occurring where the manganese excess was aggravated by the addition of 20 p.p.m. Mo. High vanadium, on the other hand, markedly reduced the phosphorus content in Exp. IV irrespective of the manganese level, a result not shown by the soybean. This fall in phosphorus was accompanied by a striking rise in iron content. In other experiments, similar treatment had produced apical chlorosis, so that some support is given to the view put forward by Millikan (1949*a*) and others, that chlorosis may be due to a failure in the utilization of iron within the plant, rather than to a reduction in uptake. Sideris & Young (1949) have since taken the matter a step further, and attribute manganese-induced chlorosis to a replacement of iron by manganese in protoporphyrin 9, the precursor of chlorophyll.

V. OATS

A single experiment was carried out with oats, var. Marvellous 6 March–4 July 1950. Treatments were confined to manganese-molybdenum relationships and were started 8 days after sowing as follows: *manganese* nil, 0·5, 5·0 and 50 p.p.m., each combined with *molybdenum* nil, 0·1, 1·0 and 10 p.p.m. Owing to the unexpected tolerance exhibited to both elements, the highest concentrations of molybdenum and manganese were both doubled on 18 April, and that of manganese further increased to 200 and 400 p.p.m. on 16 and 30 May respectively. Excellent vegetative growth was made, but though each plant produced three or four panicles, much of the grain failed to fill out properly. A deficiency of copper appeared to be the cause of the trouble, but the symptoms were recognized too late to allow of remedial measures. In consequence, the discussion of results is based on total yield and analyses made on vegetative parts only.

(1) *Interactions between manganese and molybdenum treatments*

Characteristic signs of grey-speck appeared simultaneously in all plants grown without manganese, irrespective of the molybdenum supplied, though the progress of the symptoms was somewhat retarded in the presence of 10 p.p.m. Mo. The subsequent reduction in yield, noticeable chiefly in the stem, and the delay in ripening, as shown by the percentage dry in fresh weight, were also of much the same order wherever manganese was withheld (Table 7). Plants provided with either 0·5 or

TABLE 7. *Yield and composition of oats grown with various combinations of manganese and molybdenum. (Mean of 5 plants)*

Treatment	Dry weight (g.) Leaf	Stem	Grain	Root	Total	% dry in fresh wt. Shoot	Total-N (% d.m.) Stem	P (% d.m.) Stem	Fe (% d.m.) Stem	Mn (p.p.m.) d.m. Leaf	Mo (p.p.m.) d.m. Leaf
No Mn											
No Mo	2·50	6·20	3·37	1·72	13·79	32·51	0·75	0·56	0·003	11	0·14
0·1 p.p.m. Mo	2·32	6·12	2·88	1·14	12·46	34·27	0·77	0·63	0·003	29	9·50
1·0 p.p.m. Mo	2·72	6·80	3·34	1·62	14·48	33·49	0·73	0·56	0·007	25	70·00
10–20 p.p.m. Mo	2·30	5·40	2·95	1·83	12·48	28·12	0·70	0·47	0·005	31	1600·00
Grand mean	2·46	6·13	3·13	1·58	13·30	32·10	0·74	0·56	0·005	24	420·00
0·5 p.p.m. Mn											
No Mo	2·82	9·00	3·84	1·82	17·48	43·27	0·45	0·41	0·007	38	0·18
0·1 p.p.m. Mo	2·72	11·48	6·35	1·86	22·41	42·80	0·26	0·31	0·008	24	5·80
1·0 p.p.m. Mo	2·34	10·30	6·15	1·65	20·44	41·45	0·30	0·33	0·005	30	48·00
10–20 p.p.m. Mo	2·40	9·84	6·08	1·87	20·19	42·30	0·25	0·22	0·008	27	930·00
Grand mean	2·57	10·16	5·60	1·80	20·13	42·46	0·32	0·32	0·007	30	246·00
5·0 p.p.m. Mn											
No Mo	2·92	9·42	4·96	1·93	19·23	45·02	0·51	0·34	0·005	200	Missing
0·1 p.p.m. Mo	2·38	9·04	4·30	1·19	16·91	49·08	0·45	0·35	0·013	160	5·70
1·0 p.p.m. Mo	2·78	11·10	6·93	2·22	23·03	44·57	0·28	0·38	0·012	240	56·00
10–20 p.p.m. Mo	2·52	9·92	7·22	2·14	21·80	40·55	0·22	0·23	0·003	120	830·00
Grand mean	2·65	9·87	5·85	1·87	20·24	44·81	0·37	0·33	0·008	180	(297·00)
50–400 p.p.m. Mn											
No Mo	2·70	9·64	4·43	2·52	19·29	37·90	0·47	0·25	0·02	7,100	0·41
0·1 p.p.m. Mo	2·46	10·00	4·48	1·88	18·82	37·82	0·44	0·26	0·02	8,500	6·50
1·0 p.p.m. Mo	2·90	10·62	4·86	2·58	20·96	39·30	0·37	0·27	0·02	11,500	49·00
10–20 p.p.m. Mo	2·64	9·86	5·26	2·43	20·19	36·10	0·29	0·29	0·05	6,800	1300·00
Grand mean	2·68	10·03	4·76	2·35	19·82	37·78	0·39	0·27	0·03	8,475	339·00

S.E. mean, 1·56.

5·0 p.p.m. Mn were entirely healthy, resembled each other closely and also seemed unaffected by the quantity of molybdenum present. An increase to 50 p.p.m. Mn produced some toxic symptoms in the early stages, the younger leaves turning slightly chlorotic and the roots having a pinkish tinge. Later, in spite of the increase in the manganese supplied, most visual symptoms of manganese excess disappeared, though ripening was delayed, particularly where high molybdenum had been given (Table 7). Some spare plants, subjected to 600 p.p.m. Mn when well grown, developed large reddish brown lesions on the upper leaves, starting at the centre of the laminae. Piper (1940) and Hewitt & Jones (1949) mention very similar lesions on molybdenum-deficient oats, so that Millikan's view (1948) that the symptoms of manganese excess closely resemble those of molybdenum deficiency in flax received some support. In general, however, there was no indication of an interaction between the two elements.

As regards excess molybdenum, toxic symptoms failed to appear with even 20 p.p.m. Mo. This concentration, however, had one noteworthy effect, which confirmed earlier (unpublished) work with barley. Irrespective of the manganese present, the roots of all twenty plants receiving it remained healthy during a spell of hot weather at the end of the experiment, whereas blackening occurred in all other solutions. Presumably the addition of high molybdenum had in some way improved the oxygen supply.

(2) *Nitrogen, phosphorus and iron contents*

Stem material from which the leaves had been removed was used for these analyses (Table 7). In contrast to the experiments with soybean and flax, the chief interest centred on the effect of manganese and molybdenum deficiencies on the composition of the oats. As was to be expected, a lack of either element caused a significant increase in the percentage total nitrogen content of the dry matter, particularly in the case of manganese. There was some indication that at rates of 5 p.p.m. Mn or more, utilization of nitrogen was better with the higher concentrations of molybdenum, but the level of molybdenum was without influence on the efficiency of manganese in this respect. As regards the phosphorus content, the two elements behaved differently, the percentage being increased by a deficiency of manganese, but not by a lack of molybdenum. Provided both elements were present, their concentrations were of little importance, though there were slight indications of a reduction in phosphorus content at the highest levels of either element. Although no actual chlorosis developed, the percentage of iron in the stem varied considerably with the different treatments, rising markedly as the manganese supply was increased, but not at all if the level of molybdenum was raised. In general, an inverse relationship between the iron and phosphorus content was obtained.

(3) *Manganese and molybdenum contents*

Spectrographic determinations of manganese and molybdenum were made on the leaves. The quantities of each element supplied covered a wide range and the uptakes

showed correspondingly large variations. There was, however, no indication of any consistent influence of the one element on the uptake of the other (Table 7). The grand means of the 0·5 and 5·0 p.p.m. Mn groups, for example, resembled each other as regards molybdenum content, whereas the manganese uptakes were very different. Some contamination in the no-manganese and no-molybdenum treatments appears to have occurred over the long course of the experiment, but the former was insufficient to prevent the appearance and persistence of well-marked symptoms of grey-speck disease at all levels of molybdenum supplied. The seed (average dry weight 0·049 g.) originally contained 19·3 and 0·49 p.p.m. manganese and molybdenum respectively.

VI. Discussion

The effect on soybean and flax of different combinations of manganese with molybdenum or vanadium clearly depends on the quantity of the two elements supplied. The results from the foregoing experiments may, therefore, represent an incomplete picture, and any conclusions drawn are valid only for the limited range of concentrations tested.

Replaceability of molybdenum by vanadium in the nutrition of these plants appears unlikely, in view of the contrasting results with the two elements in the presence of excess manganese and, while vanadium itself produced variable effects, they were distinct from those brought about by molybdenum. Relatively high concentrations of both molybdenum and vanadium were required before any interaction with manganese was obtained. There is thus little likelihood of the beneficial action of vanadium in counteracting manganese toxicity being due to the introduction of molybdenum as an impurity, since small quantities of molybdenum alone were ineffective.

Vanadium appears to exert some control over iron nutrition irrespective of the manganese factor, for when given in sufficient quantity it deepened the colour of the shoot and suppressed, at least temporarily, iron-deficiency chlorosis in the presence of both toxic and non-toxic manganese. The apical chlorosis that developed later, when the vanadium treatment was continued, may have been due to cumulative vanadium poisoning or to secondary effects such as reduction in iron uptake owing to restricted root growth. Some interaction between vanadium and manganese, other than that concerned with iron deficiency is, however, probable, since high vanadium reduced the leaf-crinkling symptoms that developed in soybean in the presence of excess manganese. These latter points, however, require further elucidation.

Molybdenum also seems to influence iron nutrition, but its effect on manganese-induced iron deficiency was the reverse of that of vanadium. In contrast to the results with soybean and flax no indication of a molybdenum-iron relationship was obtained with oats, but it should be noted that this crop had failed to show any leaf colour changes indicative of a disturbance in the iron balance.

Little evidence of an antagonistic action between molybdenum and manganese was forthcoming, and in oats the uptake of the one element was independent of the quantity of the other element present.

As regards nitrogen nutrition, the results emphasize the fact that a high nitrogen content is generally associated with adverse conditions, whether they be caused by deficiency or excess.

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Fig. 2

Fig. 4

Fig. 1

Fig. 3

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Millikan, C. R. (1949*a*). Lower leaf scorch of flax. *J. Dep. Agric. Vict.* **47**, 37–41.
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EXPLANATION OF PLATES 17 AND 18

(Photographs by V. Stansfield)

Plate 17

Fig. 1. Soybean. 1 p.p.m. Mn with (L–R) 0·1, 10, 20 p.p.m. Mo. No toxicity.
Fig. 2. Soybean. 1 p.p.m. Mn with (L–R) V ≡ 0·1, 1·0, 10 p.p.m. Mo. Toxicity of highest V.
Fig. 3. Soybean. 0·1 p.p.m. Mo with (L) 1·0 and (R) 20 p.p.m. Mn. Normal apex (L) compared with one showing Mn excess symptoms.
Fig. 4. Soybean. V ≡ 10 p.p.m. Mo with (L–R) 1·0, 10, 20 p.p.m. Mn. Toxicity of V throughout. Reduction of some Mn excess symptoms. (Lowest leaves have been shed.)

Plate 18

Fig. 5. Soybean. 10 p.p.m. Mn with (L–R) 0·1, 10, 20 p.p.m. Mo. Mn excess symptoms intensified with highest rate of Mo.
Fig. 6. Soybean. 10 p.p.m. Mn with (L–R) V ≡ 0·1, 1·0, 10 p.p.m. Mo. Mn excess symptoms reduced by both higher rates of V. Highest rate of V toxic.
Fig. 7. Soybean. 20 p.p.m. Mn with (L–R) 0·1, 10, 20 p.p.m. Mo. Mn excess symptoms intensified with both higher rates of Mo.
Fig. 8. Soybean. 20 p.p.m. Mn with (L–R) V ≡ 0·1, 1·0, 10 p.p.m. Mo. Mn excess symptoms reduced with highest and toxic rate of V only.
Fig. 9. Flax. R group, 1 p.p.m. Mn. L group, 25 p.p.m. Mn. Each (R–L) 0·1, 20 p.p.m. Mo and V ≡ 0·1 p.p.m. Mo. Mn excess symptoms intensified with higher rate of Mo. No toxicity of Mo with low Mn.
Fig. 10. Flax. R group, 1 p.p.m. Mn. L group, 25 p.p.m. Mn. Each (R–L) 0·1, 10 p.p.m. Mo and equivalent V. Toxicity of highest V in both groups.

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OBSERVATIONS ON THE BIOLOGY OF CLOVER ROT

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(With Plate 19 and 2 Text-figures)

The field behaviour of clover rot in eastern England indicates that infection takes place by ascospores, and not by soil-borne mycelium developing directly from the sclerotia. To support this view, experimental evidence is presented to show that cutting of red clover at the time when the ascospores are being shed, reduces the percentage infection in the following spring.

Ascospore infection of clover causes a 'non-aggressive' spotting which may develop into an 'aggressive' infection. *Sclerotinia trifoliorum* has been shown to remain viable in non-aggressive spots for at least 16 weeks. Similar symptoms occur when beans are infected with *S. trifoliorum* var. *fabae*. Non-aggressive and aggressive infections are two phases of a sequence in time, and not two alternative types of infection dependent on the initial spore load. The occurrence of non-aggressive and aggressive phases in nature explains (1) the observed delay between ascospore discharge and the appearance of obvious disease symptoms, and (2) the seasonal nature of the disease.

Aggressive infection develops only in a saturated atmosphere and within a temperature range of 5–20° C., and its establishment is favoured by a heavy dosage of inoculum. In most seasons humidity is the main 'limiting' factor; over the 18-year period, 1930–47, there is a significant regression between the severity of clover rot and the previous December plus January rainfall.

Crop rotation and autumn grazing check the disease, but the latter must be practised with discretion because, under certain conditions, its effects are more drastic than those of the disease. The only hopeful method of effective control is the breeding of resistant strains of legumes.

Introduction

The fungus *Sclerotinia trifoliorum* Eriksson is the cause of the disease that is variously known as clover sickness, clover rot, stem rot, or merely rot. The best name, however, is clover rot, the name recommended by the Plant Pathology Committee of the British Mycological Society (1944). Confusion is likely to occur if the other names are used, and the term 'clover sickness', in particular, is often loosely applied to any form of clover failure.

S. trifoliorum is a widely distributed pathogen and occurs in most countries where clover or related forage crops are grown. It has been reported from nearly every country of Europe, as well as from Russia and the North American continent. It should be noted, however, that damage ascribed to *S. trifoliorum* in Western Canada before 1934 (when Whetzel expressed the opinion that isolates of *Sclerotinia* from sweet clover in Alberta were distinct from any previously described species (Cormack,

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1946)) is now known to have been caused by *S. sativa* Drayton & Groves, the fungus most frequently associated with leguminous rots in this area. *S. sativa* has not yet been found on any host in Great Britain.

In Great Britain clover rot is widespread in East Anglia and in the chalk and limestone districts of Yorkshire; it occurs throughout eastern and south-eastern England, but is found only in scattered regions in the west country and not at all in the north or in Scotland, except as an occasional mycological record (Dennis & Foister, 1942). Garrett (1938) includes clover rot in his list of soil-borne diseases favoured by light-textured soils, quoting Pape (1931) as his authority. While it is true that the distribution of this disease over the country as a whole corresponds roughly with the distribution of chalk and limestone, it would be unwise to assume any causal relationship. In East Anglia the disease appears to be equally common on both light and heavy land, and there is no obvious connexion between the incidence of the disease and soil type.

The field symptoms of attack by *S. trifoliorum* have been described in an earlier paper (Dillon Weston, Loveless & Taylor, 1946); consequently, only a brief résumé of some of the most important points will be given here. Clover rot is sometimes noted in November or even earlier, but it is usually first seen from January to March. The outer leaves of infected plants turn olive-brown, wilt, and become covered with a white mycelium which spreads by contact to neighbouring leaves and through the petioles down to the crowns. At first only isolated plants or small patches are affected, but, if mild damp weather persists, progressively more plants become diseased so that, in extreme cases, large patches of clover completely disappear. In British literature (Amos, 1918; Wadham, 1925) these bare areas are usually attributed to the centrifugal spread of mycelium, but although spread in this way undoubtedly takes place, its importance has probably been overemphasized. The ability of mycelium of *S. trifoliorum* to creep over the soil between plants would seem, from greenhouse experiments, to be much less than that of *S. serica* Keay, yet field observations indicate that even the latter species has only limited powers of mycelial spread. Examination in 1945 of crops of *Gypsophila elegans* attacked by *Sclerotinia serica* showed that, although surface mycelium was spreading between the rows for distances up to 3 in. on either side of diseased plants (i.e. the conditions were very favourable for mycelial spread), there was only slight spread along the rows. The cultivation of *Gypsophila elegans* in rows about 15 in. apart, permits more accurate observation of the extent of mycelial spread than is possible in a field of clover. Therefore, as an alternative explanation to centrifugal spread of mycelium, it is suggested that the large patches devoid of clover represent areas in which the local conditions (either in the amount of inoculum or in the environmental conditions) were especially favourable for infection by ascospores. In this connexion, it is interesting to note that Wadham (1925), in describing an unsuccessful attempt to map mycelial spread in the field, in fact describes the disease picture that might be expected on the basis of ascospore infection. He comments on the results of his

experiment as follows: 'it is noticeable that whereas in many cases the attack starting from centres of infection spread slowly, in others the fungus made no appreciable advance: further, it is to be noted that cases of infection which are apparently new appear as late in the season as March'. These observations are readily explicable if it is assumed that infection takes place by ascospores, and that plants infected at different times during the period of ascospore discharge may rot down at various dates during the winter, in this case as late as March.

In wet, warm weather the disease establishes itself rapidly, but, in a dry frosty spell, its progress is checked for a time. Although, under suitable conditions, the fungus may kill the foliage and stems of a plant in a few days, the crown is more resistant and may escape attack when conditions are unfavourable to the fungus. It is for this reason that clover crops which seemed doomed to failure in the winter often resume vigorous growth in the spring and recover sufficiently to yield a moderate crop of hay.

If, however, the plants are killed outright, then during the spring (but also to a lesser extent during the later part of the winter) black, irregularly shaped sclerotia are produced on and in the stem bases and around the crowns of dead plants. There are no conidia produced to facilitate rapid spread. In the summer the attack dies down, and the sclerotia remain dormant in the soil until the following September, October or November, when the majority of those in the surface layers germinate and give rise to apothecia. Other sclerotia, especially those more deeply buried, remain dormant for one or more years. The ascospores are forcibly discharged from the apothecia, and are presumably carried by wind to nearby plants and neighbouring clover leys. The mode of infection of *Sclerotinia trifoliorum*, however, has long been disputed, and forms the main subject of investigation in this paper.

The host that is most commonly attacked in this country is broad red clover (*Trifolium pratense praecox*), but late-flowering red (*T. pratense serotinum*), alsike (*T. hybridum*) and white clover (*T. repens*) are also susceptible, though to a lesser degree. The disease also occurs on trefoil (*Medicago lupulina*), lucerne (*M. sativa*), sainfoin (*Onobrychis sativa*) and vetches or tares (*Vicia sativa*). The question of whether field beans (*V. faba*) are susceptible has been a matter of controversy, because there was confusion as to whether the *Sclerotinia* on this host is a normal strain of *S. trifoliorum* as found on the above-mentioned legumes or a distinct variety (var. *fabae* Keay) that is confined to beans. The author (1951), however, has presented evidence to show that the Sclerotinia disease of beans is usually caused by *S. trifoliorum* var. *fabae*, but may sometimes be due to the parent species.

Pathology of infection

(*a*) *The evidence against infection through the soil*

In the literature divergent views have been put forward as to the role played by sclerotia in the life cycle of *S. trifoliorum*. One school of thought suggests that sclerotia normally give rise to soil-borne mycelium which is the chief agency in

causing infection, and that ascospores play an insignificant part. In a recent paper from Denmark, Frandsen (1946) states: 'Thus it is my impression that the mycelium (germinating directly from the sclerotia) plays the chief part in the attack, while the ascospores are mainly concerned with the sexual propagation and the maintenance of the variation.' Wadham (1925) also suggests that there is no apparent reason why sclerotia in the soil should not produce mycelium which could, unless the competition of other soil micro-organisms was too severe, grow on vegetable detritus and subsequently cause infection. Another school of thought (Brooks, 1928) attaches great importance to the ascospores, and considers mycelial infection to be of little or no consequence. The writer shares this second view for reasons which will be given below.

During the course of this investigation many hundreds of sclerotia of *S. trifoliorum* from clover and beans have been buried at various times of the year in both soil and sand, but in no instance has the production of mycelium from these been noted. As various types of soil have been used the failure of mycelium to develop cannot be ascribed to the absence of organic matter. This is in agreement with the observations of Keay (1939), but contrary to those of Frandsen (1946). Even if a vegetative mycelium were encountered on the surface of the soil under certain conditions, the circumstantial evidence against soil-borne infection in the field is very strong, and the behaviour of the disease in the eastern counties of England (the author has had personal knowledge of the disease only in this area) agrees more closely with the view that infection is predominantly by ascospores. The lines of evidence against soil-borne infection include:

(i) Red clover is most commonly sown in the spring under a nurse-crop (usually barley or spring oats), but it *never* contracts clover rot until the late autumn or the following spring, even though at the time of sowing the disease may be establishing itself in neighbouring clover leys, i.e. the climatic conditions are presumably favourable for the establishment of the disease. If the fungus responsible were soil-borne, then young seedling clover should become infected on land known to be contaminated with sclerotia, because it is very susceptible to *S. trifoliorum* at this stage of development.

(ii) The incidence of clover rot is very dependent on the weather, and in some years the disease may be almost absent. If the fungus responsible were soil-borne, then one would expect the disease to recur to a noticeable extent each year in 'clover-sick' areas.

(iii) Field peas (*Pisum arvense*) succumb as readily as red clover to ascospore and mycelial inoculations of *Sclerotinia trifoliorum* and the variant *fabae*, and yet they are used for filling in bean crops that have been thinned by 'bean rot'. If the fungus were soil-borne, peas would be expected to contract the disease; in fact, peas affected with *Sclerotinia* have never been observed in the field in this country. The success of this practice is easily explained by the fact that peas are generally planted in March when ascospores are not being produced. It is a case of 'disease escape'.

Thus field experience of clover rot in eastern England would seem to negative the possibility of infection through the soil, although under Danish conditions, and possibly because different strains of the fungus are involved, the position may be different.

A method which suggests itself for testing the hypothesis that the ascospores are the important agents of infection is to compare the amounts of disease which develop under the same conditions in: (*a*) clover cut at different intervals during the period of ascospore discharge, and (*b*) clover not so cut. The former would be expected to show a reduced percentage of infection because, for a given amount of inoculum, there is a smaller surface area of foliage for spores to land on. An experiment on these lines was carried out by Mr R. P. Hawkins (then of the National Institute of Agricultural Botany, Cambridge), in co-operation with the author, during the season 1946–7.

The experiment was sited on land where natural infection by *S. trifoliorum* was likely to occur. The layout consisted of eighteen blocks of red clover (ten of broad red, and eight of late-flowering red), each composed of four rod-row plots, labelled from north-west to south-east as *A*, *B*, *C* and *D*. The plots were drilled in May 1946 at an approximately equal seed rate per row. The *A* plots were cut in mid-September (19 Sept. 1946), the *B* plots in early October (2 Oct. 1946), the *C* plots in mid-October (17 Oct. 1946), and the *D* plots left uncut. The number of plants per row was recorded at the time of cutting for the *A*, *B* and *C* plots, and on 30 December 1946 for the *D* plots.

Considerable retardation and thinning of the stand was observed in the cut rows, and a further establishment count was made on 14 April 1947. Clover rot was first noticed at the beginning of May but disease estimates were not made until the end of the month (29–30 May 1947), when the attack had developed and affected plants could be readily detected. The amount of infection was determined by counting the total number and the number of diseased plants in each row, the presence of the disease being confirmed on each suspected plant by a careful search for sclerotia. Most of the infected plants were killed outright but some 'grew away' from the disease on one side of the crown. These two types of diseased plants were recorded separately but, for the present purpose, may be grouped together. The results are set out in Table 1.

Table 1 shows that autumn cutting reduces the percentage of infection developing in the spring; thus cutting in mid-September reduced the percentage of clover rot from 12·2 to 1·4. This may be associated with the smaller surface area of foliage in the cut plants. The possibility that cutting had rendered the roots more resistant to invasion from soil-borne mycelium is unlikely, since the effect of cutting is to impose a check on the growth of the plants and to reduce their vigour, either temporarily or permanently. This aspect will be discussed in the section on control measures. The point proved by this experiment is that the less autumn herbage there is, the less *Sclerotinia* there will be in the following spring. The hypothesis that

ascospores are biologically important, and that in England they are the primary source of infection, would thus seem to be well established.

(*b*) *Artificial infection experiments with ascospores*

Previous workers on *S. trifoliorum* have often stated that they found it impossible to infect healthy leaves of clover by means of ascospores, but they seldom specify under what conditions. Wadham (1925) and Keay (1939) both applied an aqueous suspension of ascospores to clover leaves without result, and Munro & Ogilvie (1945) also refer to the difficulty of obtaining infection with ascospores. In the present work, ascospore infection has been induced by suspending sclerotia bearing apothecia

TABLE 1. *The effect of autumn cutting on the incidence of clover rot in the following spring (disease estimates made 29–30 May 1947)*

	Date of cutting			
	Mid-September	Early October	Mid-October	Not cut
No. of living plants at time of cutting	361	403	385	386*
No. of plants killed by *S. trifoliorum*	3	11	17	33
No. of living plants infected	2	8	10	14
Total no. of plants infected	5	19	27	47
Infection (%)	1·4	4·7	7·0	12·2

* No of plants on 30 December 1946.

about 6 in. above the experimental plants, which were kept over water under bell-jars. Although the spore load could not be controlled in this method, it was found to be most satisfactory in bringing about a generalized infection of the leaflets. Provided actively discharging apothecia were used, no difficulty was found in infecting *healthy* foliage under these conditions. Following the application of the spores, some leaves were heavily 'peppered' with infection spots but others had only a few spots. These latter were probably caused by single ascospores, for cover-glasses, placed amongst the foliage to see whether discharge had taken place, often carried only a few isolated spores.

(i) *Experiments with potted plants*

Clover. At the beginning of the second week in November 1946, when supplies of ascospores of *S. trifoliorum* were available from sclerotia buried in the spring, potted plants of broad red clover were infected experimentally under greenhouse conditions. The plants were collected the previous week from a spring-sown crop in the field. Dead or yellowing leaves were cut off at the crown, and the plants were then left in trays of water to recover from any possible effects of transplanting or trimming. Small black infection spots (Pl. 19, fig. 1) appeared 1–3 days after the spores were applied.

To observe the subsequent behaviour of the infection spots, three pots of infected plants and one control were placed in an unheated greenhouse (mean temp. 50° F.)

in a tray of water. The effect of increased humidity was observed by placing a bell-jar over one of the pots of infected plants and the control. The control remained healthy throughout the experiment and will not be referred to again. The infected plants under the bell-jar showed no further sign of infection until the last week in November (i.e. 2 weeks from the application of the ascospores), when some of the spotted leaves began to develop olive-brown, water-soaked areas (Pl. 19, fig. 2). This change was followed within 1–2 days by the appearance of tufts of mycelium which grew down the petioles and caused infection of any neighbouring leaves they touched. The mycelium was cultured and proved to be *S. trifoliorum* (on cultural characters). During December the petioles successively collapsed and the mycelium spread from plant to plant, until by the end of the month only a few leaves remained. Sclerotial formation began during the second week of December, and continued until the pot was discarded at the beginning of January 1947: by this time some of the first-formed sclerotia had already turned black.

The two pots of infected plants outside the bell-jar, however, continued to appear healthy apart from the infection spots. In view of the delay in the appearance of mycelium under the bell-jar, it was important to determine the persistence of the fungus in these spots. Portions of infected leaflets, approximately $\frac{1}{2}$ cm. square, were removed, immersed in a 1:1000 solution of mercuric chloride in 15% alcohol for periods from 1 to 6 min., washed in three changes of sterile distilled water, and then plated out on 2·5% malt agar. This treatment was attended with the difficulty that no standard time in the mercuric chloride could be consistently relied upon to give effective surface sterilization without penetration. Immersion for under 3 min. was usually insufficient to kill the surface contaminants and a copious growth of *Penicillium* or *Trichoderma* developed, whereas periods above 3 min. tended to kill the pathogen in the lesions so that isolation was sporadic. The procedure was subsequently modified by freezing the leaves overnight before dipping them in the mercuric chloride for $2\frac{1}{2}$ min.; by this means little further trouble was experienced from surface contaminants. This modification was suggested by the observation of yellow halos around infection spots on plants left in the open and exposed to night frosts. The greater ease with which the pathogen was isolated, following such treatment, is probably due to a shifting of the host-parasite balance in favour of the fungus.

Using the above techniques, *Sclerotinia trifoliorum* was successfully isolated at weekly intervals during December 1946 and January 1947. To show that spots which had remained latent for 12 weeks were not only viable on agar but also capable of inducing active infection of the host, one of the two pots of infected plants was placed under a bell-jar on 7 February. The result of increasing the humidity was the same as that obtained previously. Between 18 and 21 February most of the spotted leaves developed water-soaked areas, which spread and ultimately caused the death of the plants. Although sclerotia were formed, the mycelium was cultured as proof that *S. trifoliorum* was involved.

Weekly isolations of the pathogen from infected plants in the last remaining pot were continued during February 1947: the final isolation was made on 7 March, when the supply of material ran out. The conclusion to be drawn from this experiment, namely that *S. trifoliorum* can remain viable in infection spots for a period of at least 16 weeks, is of considerable phytopathological importance and will be discussed later.

In another experiment two pots of infected plants (also infected at the beginning of the second week in November 1946) and one control were placed, 6 days after the introduction of the apothecia, in the open on the roof of the Botany School. The infected plants showed no obvious change until the second week of December 1946 when there was a succession of night frosts. At this stage yellow halos developed around the infection spots, suggesting a renewal of activity on the part of the pathogen. *S. trifoliorum* was successfully isolated from infection spots showing such halos. A fortnight to 3 weeks later many of the petioles had collapsed, and the leaf blades came to lie on the soil. During the second week of January 1947 mycelium was seen to be developing on the laminae of many such leaves, and was spreading along the petioles and for short distances, up to $\frac{1}{4}$ in., on the soil. The mycelium was cultured and proved to be *S. trifoliorum* and not that of a secondary saprophyte.

If the previous history of these leaves had not been known and they were observed at this stage in the field, it might easily be thought that they had been infected by ascopores growing on moribund tissue. Wadham (1925), who maintained incorrectly that a saprophytic mycelium of *S. trifoliorum* must first be established before living tissue can be attacked, in fact suggests that the ascospores germinate on yellowing clover leaves and only then does the mycelium spread to the healthy plant, in a way comparable to the infection of lettuce plants by *Botrytis cinerea* (Brooks, 1908). In view of the fact that spores of *Sclerotinia trifoliorum* can penetrate mature unwounded epidermis, it seems more likely that apparent infection of clover detritus owes its origin mainly to previous infection of healthy tissue. It is doubtful whether the spores of *S. trifoliorum* could successfully compete with other micro-organisms in the colonization of clover detritus if they had not already first established themselves.

The plants were subsequently covered by snow, which rendered further observation impossible until 4 February when the snow cleared for a single day. The foliage of both the infected and the control plants had rotted down into a decaying mass, but several white sclerotia were seen on the petioles of infected plants. The snow finally cleared in the second week in March but, whereas the control plants later resumed growth, the infected plants failed to do so. Small black sclerotia were found amongst the dead remains in the two pots of infected plants.

From the observations made in these two experiments, the importance of infection spots in the field cannot be minimized. Güssow (1903) observed these spots on clover but he did not relate them to the spread of infection. It is not surprising, if only on account of their small size, that they should normally escape notice but, in addition, even when a search is made for them, it is impossible to distinguish them with any

degree of certainty from leaf spots due to other causes. On several occasions, however, the author has isolated *S. trifoliorum* from spots occurring naturally in the field, but, in relation to the number of spots tested, the number of successful isolations was very few. It is thought that this was partly due to the difficulty of effectively surface-sterilizing spotted leaves without killing the pathogen in the lesions.

If it is assumed, however, that infection in nature takes place by ascospores discharged in October and November, the fact of a delay before the disease becomes active in January or February (and sometimes even as late as April or early May) requires that the pathogen can survive the interval in infection spots. The ability of *S. trifoliorum* to survive in infection spots for periods of this order has been demonstrated in the first of the above experiments, and is again shown in the field-plot experiment that is described later. The initiation of aggressive infection depends also, in addition to the survival of the pathogen, on its ability to overcome the host resistance before its own death. On the basis of the above experimental results, it is suggested that the host resistance is broken down either by conditions especially favourable to the fungus (e.g. the prolongation of a saturated atmosphere) or by circumstances affecting the host, such as natural ageing or frost injury. It is impossible to draw a sharp distinction between these two aspects, for all external contingencies will affect both the host and the pathogen; thus, conditions favouring premature ageing of the host may not be conducive to aggressive infection because they may at the same time cut short the life of the pathogen. Considerations such as these provide a ready explanation of the sporadic nature of the disease as observed in nature.

Beans. Using the same method of infection as for the clover, two pots of bean plants (4- to 6-leaved stage) were also infected at the beginning of the second week in November 1946, with ascospores of *S. trifoliorum* var. *fabae.* Chocolate-coloured spots (Pl. 19, fig. 3), similar in appearance to those of the chocolate spot disease (*Botrytis cinerea* Fr. and *B. fabae* Sardiña), appeared 1–3 days after the spores were applied. Four days from the time the apothecia were introduced, both pots of infected plants, together with a control pot, were stood in a tray of water in an unheated greenhouse (mean temp. 50° F.). A bell-jar was placed over one of the pots of infected plants and over the control. The subsequent events were similar to those in the clover experiment. The control plants remained healthy, but the infection spots of the infected plants under the bell-jar coalesced in the course of a few days, causing a blackening of the leaf tissue (Pl. 19, fig. 4). During the second week of the experiment tufts of mycelium appeared which spread rapidly and caused the collapse and death of the plants. It will be noted that, under the conditions of this experiment, the aggressive mycelium was established more quickly on the bean foliage than on the clover foliage.

The infected plants in the pot outside the bell-jar continued to grow but, apart from some slight coalescence between lesions, no further disease symptoms developed.

Ten weeks after the spores were applied this pot was covered with a bell-jar, and a week later mycelial threads were evident on some leaves. On other infected leaves no mycelium appeared, suggesting that in these the fungus had died out. The mycelium spread rapidly, forming sclerotia in its progress, and ultimately killed all the plants.

In the literature there is no precise picture of the early symptoms of the Sclerotinia disease of beans (or bean rot as it is commonly called) in the field, and the disease is first noticed with the wilting and death of the lower leaves and the lower part of the stem. These symptoms are not to be confused with injury due to frost, which also causes blackening at the collar with wilting and death of the primary shoot. The blackening due to frost injury is a definite zone at soil level, the root remains sound and when pulled up the upper portion of the plant breaks off. In contrast, the blackening caused by *Sclerotinia trifoliorum* var. *fabae* is slimy and soft to the touch, and when the plant is pulled the root comes up with the rest of the plant and is blackened. The fact that beans behave in a comparable manner to clover when artificially infected with ascospores, strongly suggests that *Sclerotinia* infection of beans can also be divided into non-aggressive and aggressive stages. Winter spotting of bean leaves is always attributed to the chocolate spot disease (*Botrytis cinerea* and *B. fabae*), but it is quite possible that some of this spotting is caused by ascospores of *Sclerotinia trifoliorum* var. *fabae*. The author has not been able to isolate *Sclerotinia* from chocolate-coloured spots on bean leaves in the field, but the evidence available would certainly indicate that such a situation holds. In addition, *S. trifoliorum* var. *fabae* has been shown to remain viable in non-aggressive spots for at least 10 weeks, which is longer than the delay between ascospore discharge (the times of apothecial production in *S. trifoliorum* var. *fabae* are the same as in *S. trifoliorum* proper, namely the late autumn) and the appearance of the disease in years when the disease is noted early. In 1945 severe attacks of bean rot were noted as early as January. Circumstantial evidence, similar to that in favour of ascospore infection in clover, also supports the case for non-aggressive and aggressive infection by *S. trifoliorum* var. *fabae*; in particular, it may be noted that spring-sown beans do not contract bean rot.

(ii) *Field-plot experiments*

Infection experiments were carried out in the winter of 1947–8 on a field plot, measuring approximately 5 × 10 yd., put down to broad red clover. The clover was sown, in accordance with usual farm practice, at the beginning of the previous April between rows of barley that had been sown a fortnight earlier. The barley was harvested by hand in July, and the stubble was left in the ground.

On four successive occasions, at fortnightly intervals between mid-November and the end of December 1947, growing plants in areas of 4 sq.ft. were artificially infected by ascospores. On each occasion two areas, one on each side of the plot, were chosen, thus giving eight treated areas in all. The plants were infected by

enclosing them, along with six apothecia suspended on sticks, under metal frames, 2 × 2 × 2 ft., with sheet zinc sides and a glass top. The frames and apothecia were removed after 3 days, except on one area (one of the two areas infected in mid-December) where the frame was left for a fortnight in order to maintain the humid conditions inside for a longer time. Although the plants in all eight treated areas were heavily peppered with infection spots at the time that the frames were removed, the disease failed to develop in five of them. The three areas in which the disease did develop comprised the one over which the frame was left for a fortnight, and the two to which spores were applied at the end of December. In the former area two plants were killed outright shortly after the frames were removed, but the disease did not spread or reappear on other plants. In the latter two areas the disease was not evident until mid-April 1948, when several plants became infected from mycelium developing on spotted leaves which had become moribund. A few plants were killed but the progress of the disease was subsequently checked by the advent of warm dry conditions at the end of April. No disease developed on the remainder of the plot, which acted as a control.

Although this experiment was characterized by the almost complete failure of aggressive infection to develop, owing to the season being unfavourable for clover rot, the delay of $3\frac{1}{2}$ months in the appearance of disease in the two areas mentioned above, is consistent with the delay observed in many seasons between the period of ascospore discharge and the time when the disease is first noted. Certain workers (Munro & Ogilvie, 1945) have questioned whether the ascospores are the infective agents on the grounds that outbreaks of clover rot may be few in number and slow to appear in the neighbourhood of fields where ascospores are known to have been shed, and also that infection may not occur when conditions are favourable to other leaf-spotting fungi, such as *Stemphylium*, *Pseudopeziza*, *Dothidiella* and *Uromyces*. These objections, however, overlook the distinction between non-aggressive and aggressive infection. It is postulated that, if the air is charged with ascospores, then non-aggressive infection will normally take place, but that the time and extent to which the aggressive phase develops will be conditioned by the subsequent weather. It must be remembered that the development of aggressive from non-aggressive infection in *Sclerotinia trifoliorum* requires prolonged periods of high humidity and not the relatively short periods which are apparently sufficient for other leaf-spotting fungi of clover. It is only the aggressive stage that causes damage and, in seasons when conditions do not favour the development of aggressive infection, widespread spotting may be of no consequence. The above objections, therefore, far from being incompatible with ascospore infection, may in fact be regarded as strong evidence in favour of the occurrence of ascospore infection with non-aggressive and aggressive phases.

(*c*) *Phases of infection*

The experimental infection of clover and beans with ascospores has shown that infection by *S. trifoliorum* can be divided into two phases, 'non-aggressive' and

'aggressive'; also that infection does not develop beyond the non-aggressive phase unless the host is maintained in, or returned to, an atmosphere approaching saturation.

The ability of a pathogen to remain latent for a greater or lesser length of time after initial infection is a concept that has been largely neglected to date in plant pathology, although due consideration has been paid to it in animal pathology. Baker (1938) has shown that certain storage rots of tropical fruits (grapefruit, mangoes, papaws, etc.), especially those caused by *Colletotrichum gloeosporioides* (Penzig) Sacc., develop from infections that became established during the setting of the fruit in the field. The fungus is arrested at a very early stage, and infection is confined to the superficial layers of the skin. These latent infections do not normally develop further until the fruits begin to ripen in storage. Apart from these storage rots the author knows of no other authenticated cases in which the position is strictly comparable to that found in *Sclerotinia trifoliorum*, although it seems likely that latent infection is probably not a rare phenomenon. In animal pathology the concept is fully recognized; it is well known, for instance, that once herpes infection has taken place the pathogenic agent may persist in the body throughout life, but that it will only assume a virulent form when stimulated into action by one or other of a variety of causes. A similar situation is found in *S. trifoliorum*, where the restoration of a high humidity has been shown to incite the aggressive phase in a plant in which infection had remained non-aggressive for 12 weeks.

In plant pathological literature the recognition of non-aggressive and aggressive infection has been confined to a few diseases caused by species of *Botrytis*. Wilson (1937) was the first to use the terms 'non-aggressive' and 'aggressive' when he used them in describing the symptoms of the chocolate spot disease of beans. They have since been applied to other diseases. Fruit spot of tomato, which was previously attributed to non-parasitic causes or to insect injury, has now been established (Ainsworth, Oyler & Read, 1938) as a non-aggressive infection of *B. cinerea* Fr., which also causes the aggressive grey mould disease of the same host. Similarly, tulip fire, caused by *B. tulipae* Lind, also occurs in aggressive and non-aggressive forms (Beaumont, Dillon Weston & Wallace, 1936). Brooks (1939) has also described a similar situation in roses. The most complete information on this subject applies to the chocolate spot disease of beans, which was intensively studied by Wilson. He showed that if a dilute suspension of *Botrytis* spores is sprayed on bean foliage, discrete brown spots develop; these spots increase only slightly in size with the passage of time, even though the host is maintained in an environment suitable to infection. If, on the other hand, a dense spore suspension is applied the lesions coalesce and, provided humid conditions are maintained, mycelium appears on the surface and the foliage is killed. In chocolate spot, therefore, aggressive and non-aggressive infections are considered as two alternative types of infection dependent upon the dosage of inoculum, and not as two phases of a sequence in time as in *Sclerotinia trifoliorum*. For this reason, attention has been paid in the present work to spore load as a possible factor in the development of aggressive infection.

For the purpose of defining the external conditions necessary for the development of the aggressive phase, three variable factors have been studied, namely temperature, humidity and spore load. Their effects have been studied on detached leaflets of *Vicia faba*, which were used in preference to those of *Trifolium pratense* because they survive longer when detached from the plant. The leaflets were infected by suspending mature apothecia for 12 hr. (9 a.m. to 9 p.m.) above bean plants (4- to 6-leaved stage) which were maintained over water under bell-jars in a heated greenhouse. Although the spore load could not be controlled in the same way as in Wilson's experiments, light and heavy infections were obtained by suspending the apothecia at different heights above the plants. Infected leaflets were classified as heavily or lightly infected, according to whether they had more than fifty or less than twenty spots. After the apothecia were removed the plants were left for a further 42 hr.; this interval was considered sufficient to allow all the potential lesions to develop, and also permitted the removal of infected leaflets at a time when their carbohydrate content was at a high level, i.e. 3 p.m. By removing the leaflets at 3 p.m. it was thought that their life would be prolonged in the detached state. The detached leaflets were laid in Petri dish lids which were placed in the dark over humidity control solutions (NaCl was used) at different temperatures. Five infected leaflets, plus a healthy leaflet as a control, were used for each of the twenty treatments (see Table 2). The experiment was repeated, and both experiments were continued for 16 days.

Table 2. *The development of the aggressive phase.* (*Average number of days subsequent to infection for the invasion of an entire leaflet of* Vicia faba)

Relative humidity (%)	Spore load	Av. no. of days for invasion of leaflet at 1° C.	5° C.	15° C.	20° C.	25° C.
100	Heavy	—	11	6	5	—
	Light	—	(15)	(8)	(8)	—
97·5	Heavy	—	—	—	—	—
	Light	—	—	—	—	—

— = aggressive phase did not develop.
( ) = only some of the infected leaflets in the series developed an aggressive infection.

The aggressive phase failed to develop during the 16 days that the experiments were continued in fourteen out of the twenty treatments. In the six treatments in which the aggressive phase did develop, there was considerable variation between the individual leaflets of any one treatment in the time taken for the aggressive phase to develop. Table 2 records the average time per treatment (each figure represents the average of ten leaflets) for the mycelium to invade an entire leaflet. The figures for the light spore load infections at 100% R.H. are included in brackets because the aggressive phase developed on some only of the leaflets. If the spots were widely separated from one another then they merely increased slightly in size,

but if two or more spots were in proximity then they coalesced to form an aggressive infection which attacked the rest of the leaflet.

From Table 2 it will be seen that, under the conditions of the experiment, the aggressive phase developed only in a saturated atmosphere and within a temperature range of 5–20° C., also that its establishment is favoured by a heavy dosage of inoculum. The fact that a single lesion of *Sclerotinia trifoliorum* is unable to initiate the aggressive phase does not mean that the aggressive phase should be interpreted primarily in terms of the dosage of inoculum as in *Botrytis cinerea*. The number of lesions per leaflet figured in Wilson's paper for non-aggressive infection would very definitely have been sufficient to have induced an aggressive infection if they had been caused by *Sclerotinia trifoliorum*. Although it is convenient to regard the non-aggressive and aggressive phases in *Botrytis cinerea* as two alternative types of infection, and in *Sclerotinia trifoliorum* as two phases of a sequence in time, it is suggested that the difference is really one of degree rather than of kind. The two situations may be regarded as two manifestations of the same phenomenon, the apparent difference being associated with the different pathogenicities of the two fungi.

(*d*) *Germination and viability of the ascospores*

The successful establishment of clover rot depends on the ability of the pathogen to initiate infection, and then to maintain itself until such time as the environmental conditions are favourable for the onset of the aggressive phase. It has already been shown that, once infection has been achieved, *S. trifoliorum* can survive in the form of non-aggressive spots for periods up to 3 months; it now remains to study the germination and viability of the infective agents, namely the ascospores.

(i) *The influence of temperature on spore germination*

Spore germination has been most conveniently studied with ascospores collected on cover-glasses; no adhesive substance is necessary in the collection of the ascospores, for the spore surfaces at the time of discharge are mucilaginous and cause the spores to adhere to the glass substratum. The cover-glasses were suspended about ½–1 cm. above actively discharging apothecia for a maximum period of 10 min. and then placed, spores downwards, on glass rings in Petri dishes containing a shallow layer of distilled water. The Petri dishes were immediately transferred to controlled temperature chambers. In the course of a few minutes condensation took place on the cover-glasses so that the spores were maintained in the presence of liquid water. By this technique spores were obtained undamaged and as nearly comparable as possible with natural infective material.

Counts were made of the percentage germination of ascospores held at 0, 1, 5, 15, 22·5 and 27° C. The counts could not be made at the experimental temperatures, and therefore the method of periodically counting the percentage germination of the same spore populations had to be discarded. Transference of the spores to the warm laboratory, even for periods as short as 10 min., might have

significantly increased the germination of spores held at the lower temperatures. Instead, several spore populations were left uninterruptedly at each temperature, and removed one by one at the end of varying time periods. After removal, each population was immediately fixed in formal-acetic-alcohol and subsequently stained in cotton blue in lactophenol. Germination counts were made in microscopic fields taken at random, the process of counting being facilitated by a cross-hatched disk in the eyepiece of the microscope.

TABLE 3. *The influence of temperature on ascospore germination*

Temp. (° C.)	Time (hr.)	Germination (%)	Temp. (° C.)	Time (hr.)	Germination (%)
0	6	0·0	15	1	0·0
	12	0·0		2	4·0
	24	0·0		2·5	53·6
	48	0·0		3	79·2
1	2	0·0		3·5	84·8
	4	0·0		4·5	88·0
	6	0·0		6	99·2
	7	0·0		8	98·8
	8	22·4		24	100·0
	9	60·8	22·5	1	0·0
	11	65·6		1·5	0·0
	24	90·0		2	5·6
	48	89·2		2·5	28·0
5	2	0·0		3·5	47·2
	3	0·0		4	80·0
	3·5	33·2		6	92·0
	4	80·0		24	94·0
	4·5	83·2	25	2	0·0
	6	77·6		4	0·0
	8·5	94·0		6	33·2*
	12	100·0		24	42·4*
	24	99·6	27	6	0·0
				12	0·0
				24	0·0

* Abnormal germination.

The results are shown in Table 3. Each germination percentage was based on a count of 250 spores. Germination occurred under the conditions of the experiment through a temperature range of 1–25° C. At 15° C. germination commenced in $1\frac{1}{2}$–$2\frac{1}{2}$ hr. and was complete in 6–8 hr. At 1 and 5° C. germination was delayed, though at 5° C. no actual inhibition of germination occurred. The final percentage of spores germinating at 1° C. was reached only after 24 hr., and was significantly less than at 5 and 15° C. No germination occurred at 0° C. even after 48 hr. At 22·5° C. spores germinated readily, but the final value was significantly less than at 15° C. At 25° C. germination was low in frequency and the germ tubes were abnormal in appearance. Above 25° C. the spores were killed.

(ii) *The influence of humidity on spore germination*

The fact that the author has found ascospores to cause infection readily when the host plants are stood over water under bell-jars, whereas previous workers have failed to obtain infection, strongly suggests that germination of ascospores is dependent on the continued presence of a film of water on the leaf surface. To test this conclusion, spores, collected on glass-slides, were enclosed in ground-glass stopper jars maintained at a uniform temperature (15° C.) and containing solutions of sodium chloride to regulate the relative humidity.

The data of this experiment are summarized in Table 4. No germination had occurred after 1 week at humidities lower than 95·1%, but at 97·5 and 98·8% R.H. distorted germ tubes, which failed to develop beyond 5–10μ long, appeared after 2–3 days (N.B. at the same temperature in the presence of liquid water germination commences in $2\frac{1}{2}$–$3\frac{1}{2}$ hr.). Normal germination occurred at saturation, but condensation inevitably occurred at this point owing to slight temperature fluctuations of the chamber.

TABLE 4. *The influence of humidity on ascospore germination*

Relative humidity (%)	No. of g. NaCl per 100 ml. H_2O	Germination (%)
100	0	98·4
98·8	2	Approx. 95% abnormal
97·5	4	Approx. 40% abnormal
95·1	8	0·0
92·6	12	0·0
90·2	16	0·0

In spite of the occurrence of abnormal germination at 97·5 and 98·8% R.H., these results support the conclusion that liquid water is necessary for germination.

(iii) *The influence of temperature and humidity on infection*

Having studied the effects of temperature and relative humidity on spore germination *in vitro*, an experiment was made to determine the effect of these two factors on the establishment of the pathogenic relation. The technique was essentially the same as before, except that a bean leaf was substituted for a glass-slide as the germination surface and only the presence or absence of infection was recorded.

Successful infection was achieved over the same range of conditions (at 100% R.H. and between 1 and 22·5° C.), but, whereas infection took place within 8–12 hr. at 15 and 22·5° C., infection spots were not evident at 5 and 1° C. for 2 and 4 days respectively.

(iv) *The viability of the ascospores*

It is possible that the delayed appearance of clover rot in some seasons is due to the ascospores being able to survive periods of unfavourable weather. The viability of ascospores in relation to adverse conditions has been studied by allowing the

ascospores to dry out. Ascospores were collected on sterilized slides and stored in Petri dishes at room temperature (16–18° C.) in diffuse daylight. At daily intervals these were set up to germinate. The few observations made indicated that the resulting percentage germination falls off steadily to zero after desiccation for 5–6 days under these conditions. The matter was not pursued because it seemed clear that ascospores are short-lived and do not survive periods of unfavourable weather in nature.

Field problems

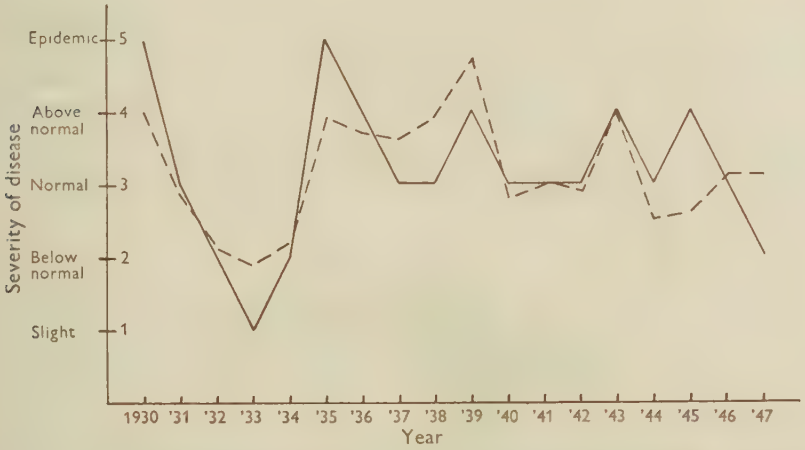
(*a*) *Environmental conditions which favour epidemics*

It is well known, on observational grounds, that the prevalence and intensity of clover rot depend in large measure on weather conditions. In published accounts of the disease, however, the relation between weather and disease intensity is defined only in general terms, such as that clover rot appears to be worse after a mild winter. Further, no explanation is offered to account for this relation.

In view of the demonstration of non-aggressive and aggressive phases in the life cycle of *S. trifoliorum*, the most reasonable hypothesis to account for the wide seasonal variation in the severity of clover rot, is that the weather subsequent to infection determines the extent to which the aggressive phase develops. It will be recalled that it is only the aggressive stage which causes damage and that widespread infection, provided it remains non-aggressive, may in itself be of no consequence. Under natural conditions the ascospores of *S. trifoliorum* are disseminated mainly in October and November, but the disease is not usually evident until the end of the following January or early February. The environmental conditions prevailing during the intervening months of December and January are therefore likely to be critical in deciding to what extent the disease shall develop.

Two factors of the environment may be considered, namely, temperature and rainfall. It has been shown in an earlier section of this paper that the aggressive phase will develop only within a temperature range of approximately 5–20° C. and in an atmosphere approaching saturation. The influence of temperature and humidity on the lateral extension of mycelium of *S. trifoliorum* and *S. trifoliorum* var. *fabae* is also known. Keay (1939) found the minimum, maximum and 'optimum' temperatures for linear spread of mycelium of both these fungi to be 0, 27 and 20° C. respectively. The author (1948) has shown, for the same two fungi, that active mycelial growth takes place only within a humidity range of approximately 95–100% R.H., growth being completely inhibited at 90·2% R.H. in both fungi. In contrast to a rust fungus, for example, environmental humidity subsequent to infection is important in *S. trifoliorum*, for here the mycelium is not entirely endophytic but spreads also on the surface of the host. For the reason that aggressive infection and active mycelial growth occur over a wide temperature range but only within narrow limits of humidity, rainfall may be expected to be more decisive than temperature in the epidemiology of clover rot.

For the purpose of establishing the dependence of clover-rot severity in any one year on the previous December-January rainfall, data applying to East Anglia were collected for the years 1930 to 1947 inclusive. East Anglia was selected because *S. trifoliorum* is probably more generally distributed in this area than elsewhere in England. Averages of the December plus January rainfall at ten stations distributed throughout this part of eastern England were obtained from the monthly summaries of the Meteorological Office. The severity of clover rot for the corresponding years was classified as epidemic, above normal, normal, below normal and slight. This arbitrary scale is based on the categories adopted in the 1933–42 report of the Ministry of Agriculture and Fisheries (Bulletin no. 126). Most of the information used



Text-fig. 1. The observed estimates of clover-rot severity in East Anglia for the years 1930–47 plotted against values calculated from the rainfall data of the preceding December and January. ——, disease severity observed; – – –, disease severity predicted from the December plus January rainfall.

in assessing the severity of the disease in the different years has been kindly supplied by Dr W. A. R. Dillon Weston, the Advisory Plant Pathologist to the eastern province. The disease estimates have been confirmed by Mr W. C. Moore, who states that they are in very close agreement with the records at the Ministry's Plant Pathological Laboratory, Harpenden.

Statistical treatment of the data (the disease estimates have been scored 5, 4, 3, 2, 1 to correspond with epidemic, above normal, normal, below normal and slight) shows a significant regression between disease severity and December plus January rainfall; $b = 0{\cdot}01872 \pm 0{\cdot}004245$, $t = 4{\cdot}4$. The regression equation is $y = 0{\cdot}01872x + 1{\cdot}1397$, where y is the disease severity that may be expected for a given value, x, of the December-January rainfall. In Text-fig. 1 the observed disease estimates for the years 1930–47 are compared with the expected values calculated from the rainfall

figures. It will be noted that there are only two years, namely 1945 and 1947, in which there is a marked disparity between the observed and expected values. In view of the arbitrary nature of the disease estimates, the dependence of disease severity on December-January rainfall would seem to be all the more striking.

Although there is therefore a definite correlation between rainfall and severity of clover rot, it is not suggested that rainfall is the only decisive factor. Foister (1929) has pointed out the difficulty of separating the relations of temperature and rainfall, and suggests that the severity of certain diseases in damp weather may be due primarily to the favourable temperatures which often accompany it. Examination of the temperature records for the period under review, however, shows that the mean temperatures for December and January do not undergo the wide seasonal fluctuations in East Anglia that rainfall does. There is no significant regression between disease severity and the mean temperatures for the months of December, January and February, considered either singly or jointly. From these considerations it is postulated that the establishment of clover rot in most seasons is primarily conditioned by the December-January rainfall, but that other factors, such as the extent to which the initial non-aggressive infection took place, contribute to the intensity attained in any given year.

(*b*) *Control measures*

The control of clover rot presents problems common to both soil-borne and air-borne pathogens. The subject will be discussed under the following heads: (i) crop rotation, (ii) disease control under the growing crop, (iii) resistant varieties.

(i) *Crop rotation*

Clover rot is a disease that is essentially linked with a rotation in which an autumn-sown legume is grown at least once in 4 years, a practice that provides conditions favourable for the progressive contamination of the land by sclerotia. A field which contains more than a small population of sclerotia in the surface layers will almost certainly suffer from an attack of *Sclerotinia* if susceptible crops are grown and the weather is normal. Gilbert (1901) found that after growing clover on the same plot of ground for many years there came a time when he could not get it to grow at all. At this time he was able to collect 6 oz. of sclerotia from 10 sq.yd. of ground. Pape (1937) has reported that sclerotia of *S. trifoliorum* survived in soil for $7\frac{1}{2}$ years, but this is probably near the upper limit of longevity, because 8-year rotations (in which susceptible crops are kept off the land for seven years) are usually sufficient to give adequate control of the disease on contaminated land. There is, however, no precise information on the survival of sclerotia of *S. trifoliorum* in soil, but soil temperature, moisture, reaction, organic content and microbial activity are likely to be determining factors.

Until recently crop rotation was thought to be the primary consideration in the control of clover rot. During the second World War, however, when thousands of

acres of pasture were ploughed up, many cases of severe clover failure were observed that could not be explained on the previous cropping. One striking case, seen by the author in 1945, was at Shelton, Norfolk. The field had been tumble-down grassland, widely colonized by bushes of *Rubus fruticosus*, for at least 30 years, until it was ploughed up for a crop of oats in 1940. This was followed by a succession of white-straw crops until 1944 when broad red clover was sown under barley. In spite of the fact that no leguminous crop had previously been taken for at least 35 years, the 1945 clover crop was a 50–60% failure due mainly to clover rot. An outbreak such as this could be readily explained if it were shown that *Trifolium repens*, the principal clover of grassland, is commonly attacked by *Sclerotinia trifoliorum* in the field. In the absence of any evidence to support such a possibility, however, it cannot be assumed that the soil was heavily contaminated with sclerotia before ploughing.

It is clear from experience gained in this way that crop rotation is only a palliative measure, and the effect of a sound rotation may be nullified if heavily infected fields occur in the vicinity. This does not minimize the importance of a proper rotation in checking the increase of the inoculum, but emphasizes that effective control must also be sought along other lines. When clover has been severely attacked, the old ley should be ploughed in deeply (if this is possible) before October, in order to bury the sclerotia at a depth sufficient to prevent the formation of apothecia; sclerotia will not form apothecia if buried deeper than about 2 in. (Dillon Weston *et al.* 1946).

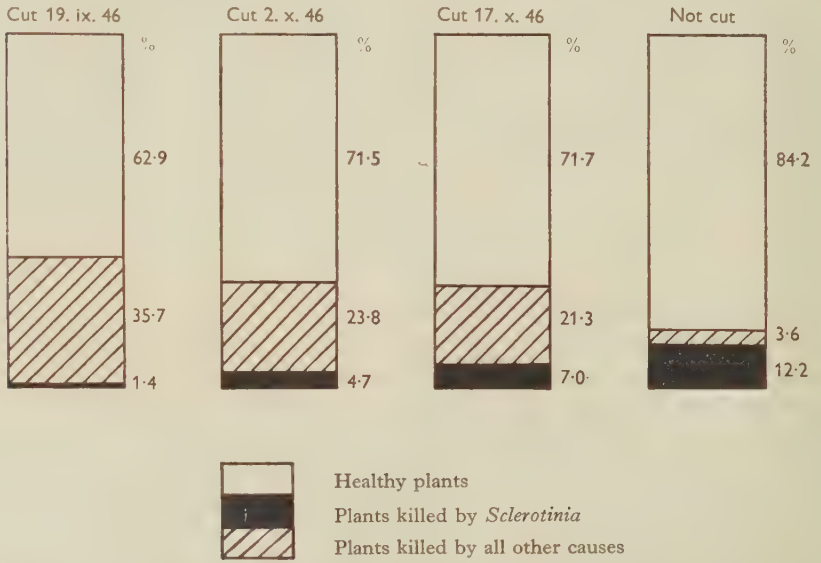
(ii) *Disease control under the growing crop*

The control measures to be considered under this heading arise from the fact that infection by *S. trifoliorum* is dependent on air-borne ascospores; they aim at preventing the germination of the ascospores on the leaf and stem surfaces.

The most obvious method that might be effective is the application of fungicidal sprays and dusts. Various attempts, using both copper and sulphur compounds, have been made (e.g. Wadham, 1925), but the results have been inconclusive. Also the recent claim from the Lyngby Experimental Station, Denmark (Gram, 1944) of the successful control of clover rot by successive dustings with pentachlor-nitro-benzene, has not been substantiated in this country. It is the opinion of the author that, although fungicides might be applicable on a small scale, their use would be both uneconomic and impracticable for field crops.

The most promising method of disease control under the growing crop is to graze the clover at a time when the ascospores are being discharged. Further, the practice of grazing is more likely to find favour with the farmer because it does not interfere with his established routine to the same extent that any spraying or dusting programme does. There are several observations (Dillon Weston *et al.* 1946) which indicate that grazing of clover by sheep between September and November may reduce the intensity of clover rot and prevent its spread, but these refer only to isolated fields. Although the beneficial effect of grazing would seem evident in such cases, it is impossible to assess the disease picture that would have developed in the absence of

grazing. More convincing evidence, however, applicable to a large area, has been obtained in recent years from Yorkshire. During the second World War an area of the Yorkshire Wolds, amounting to approximately 100,000 acres, was taken over by the War Department for tank training. As a consequence of this all the sheep and other stock, other than that kept indoors, had to be sold from the farms compulsorily. This meant that the clover crops were not grazed in the normal manner, it being customary in this area to graze clover in the first autumn of its growth if it shows vigorous growth. The interference with this grazing practice resulted in greater losses due to clover rot in the crops throughout the area than had been encountered previously. The writer is indebted for this last piece of information to Mr R. Holliday, formerly Technical Officer to the East Riding of Yorkshire.



Text-fig. 2. The effect of autumn cutting of seedling red clover on plant establishment in the following spring.

That a reduction in the amount of leafage during the period of ascospore discharge does in fact alleviate the incidence of clover rot has been shown in an earlier section. The question therefore arises as to the desirability of grazing (or mowing) as a routine preventive measure. In this connexion it is relevant to refer to the experiment, previously described, on the effect of autumn cutting on seedling red clover. In this experiment, in addition to estimating the *Sclerotinia* damage, account was also taken of plant establishment and yield of hay. The results are interesting because they show that in this experiment the control measure was more drastic than the disease, i.e. the diminution in plant establishment due to cutting far outweighed the loss caused by *Sclerotinia* (Text-fig. 2). This result is even more strikingly shown

by the yield figures (Table 5). The severe check to the cut plants may have been a seasonal effect due to the severe winter of 1946–7, and in another year there might not have been the same severe loss incurred by cutting. The results emphasize, however, the importance of management in the cultivation of clover and therefore the extreme risk in grazing as a possible control measure. Until more is known about successful clover management, the practice of grazing to control clover rot should be carried out with discretion because, under certain conditions, it will defeat its own end.

TABLE 5. *The effect of autumn cutting of red clover on the subsequent yield*

Treatment	Total yield (in oz.) per treatment
Cut mid-September (19 Sept. 1946)	453
Cut early October (2 Oct. 1946)	633
Cut mid-October (17 Oct. 1946)	596
Not cut	1090

(iii) *Resistant varieties*

The counsel of perfection in the control of any air-borne disease is the production of varieties resistant to attack. In the case of clover rot, their production is particularly desirable because there is as yet no satisfactory alternative method of control. The problem is complicated by the fact that the only type of clover which can be included in the typical four- and five-course rotations, namely, broad or early-flowering red clover (*Trifolium pratense praecox*), is the most susceptible of all British clovers. The late-flowering type (*T. pratense serotinum*) is more resistant, but cannot be used for the purpose of a '1 year' ley in a four- or five-course rotation. Further, the work of selecting resistant strains of clover was for many years handicapped in this country by the sporadic nature of the disease, with the consequent difficulty of submitting likely material to the necessary severe conditions of infection. In years when there was only a slight infection no selections could be made as the experimental material had obviously escaped attack. The Welsh Plant Breeding Station at Aberystwyth has now developed a technique for the artificial inoculation of large numbers of seedlings by means of mycelial infection. Watkin Williams, who was until recently in charge of this work, has summarized (1950) the present position with regard to the breeding of red clover species for resistance to *Sclerotinia trifoliorum*.

The work described in this paper was carried out under the supervision of Prof. F. T. Brooks, C.B.E., F.R.S., to whom I should like to express my sincere thanks for the advice and constructive criticism which he gave throughout the course of the investigation. I am also deeply indebted to Dr. W. A. R. Dillon Weston, who suggested the problem to me. Thanks are also due to Dr F. Raw for discussing the presentation of some of the data, and to Mr F. T. N. Elborn for taking the photographs.

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EXPLANATION OF PLATE 19

Fig. 1. *Sclerotinia trifoliorum* on *Trifolium pratense*. 'Non-aggressive' spotting.

Fig. 2. *S. trifoliorum* on *Trifolium pratense*. 'Aggressive' phase.

Fig. 3. *S. trifoliorum* var. *fabae* on *Vicia faba*. 'Non-aggressive' spotting.

Fig. 4. *S. trifoliorum* var. *fabae* on *Vicia faba*. 'Aggressive' phase.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

EFFECTS OF CULTURAL TREATMENTS ON WHEAT AND ON THE INCIDENCE OF EYESPOT, LODGING, TAKE-ALL AND WEEDS. FIELD EXPERIMENTS 1945–8

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(With Plate 20 and 11 Text-figures)

An experiment was made on the fourth, fifth and sixth successive crops of winter wheat to determine the effects of various treatments on the troubles which result from close cereal cropping. Eyespot and lodging were prevalent in the first year (1946); weeds in the second; eyespot, lodging, take-all and weeds in the third.

Spraying with H_2SO_4 reduced the incidence of eyespot, lodging and weeds, and increased yield of grain on plots which received sulphate of ammonia (by 2·7, 2·2 and 10·0 cwt./acre in successive years).

Sulphate of ammonia increased the incidence at harvest of eyespot and lodging, reduced take-all and consistently increased yield of straw. Eyespot and lodging reduced the effect of the fertilizer on yield of grain, take-all increased it.

Increase in seed rate increased the incidence of severe eyespot and of take-all; it increased lodging except when plants were dwarfed by take-all.

Weight of straw and percentage straws with severe eyespot lesions independently affected lodging, together accounting for 51 % of the variance in percentage area lodged at harvest and 64 % of that lodged 33 days earlier.

Mean yields of grain on untreated plots sown with 3–3½ bushels seed/acre fell from 26·0 to 22·5 to 11·7 cwt./acre in successive years, whereas yields of 28·4, 29·9 and 29·1 cwt./acre were obtained on sprayed plots sown with 1½–2 bushels seed/acre which received 4 cwt./acre sulphate of ammonia, showing that high yields were maintained when eyespot, lodging, take-all and weeds were controlled.

By 1948 yields of grain on unsprayed plots had fallen to the level of those on similarly manured plots on the continuous wheat experiment on Broadbalk field. Spraying increased grain by amounts similar to those resulting from one year's fallow on Broadbalk; but fallow had its greatest effects on plots with low nitrogen, spraying on those with high nitrogen.

INTRODUCTION

Such a high proportion, in some counties as much as 40 % of the arable land, is devoted to wheat and barley that often not more than a year separates these crops. This is not enough to control eyespot caused by *Cercosporella herpotrichoides* Fron., which is prevalent in wet seasons, causing serious loss, on much of the heavier land of Britain (Glynne & Moore, 1949). Where winter wheat is taken in consecutive years weeds increase and take-all, caused by *Ophiobolus graminis* Sacc., is often serious, especially on light land. It is therefore important to understand how cultural treatments affect crops infested by these diseases and by weeds, as well as how they affect healthier crops, and to determine whether the losses which so often result from close cereal cropping can be reduced by varying cultural methods.

The present paper summarizes the effects of various treatments, in a factorially designed field experiment on heavy land, on the fourth, fifth and sixth consecutive crops of winter wheat, following one heavily infected and severely lodged by eyespot. In the first year of the experiment eyespot and lodging, in the second weeds and in the third eyespot, lodging, take-all and weeds were very prevalent on control plots. Effects of treatments on plant, shoot and straw numbers, on disease and weed incidence, on lodging and yields are here recorded and some of the effects correlated.

Treatments, selected from those noted as being promising in field surveys and pot experiments included:

(1) *Spraying with* H_2SO_4 to reduce infection by eyespot and so make it possible to compare the effects of other treatments at two levels of disease intensity. The spray was applied both in autumn and early spring because this combination had previously given the greatest control (Dion, 1943).

(2) *Sulphate of ammonia* applied in early spring generally increased the percentage straws severely infected by eyespot at harvest in the field but always reduced it in pot experiments (Glynne, Dion & Weil, 1945). In the field, the fertilizer, by increasing foliage, helps to maintain a damp atmosphere round the plant bases and this favours development of the fungus. In pot experiments this effect is masked by frequent watering, but the extra tillers produced on better nourished plants, protect and delay penetration of the central ear-bearing straws.

(3) *Rate of seedling*. Sprague & Fellows (1934) in America and Oört (1936) in Holland observed that lowering seed rate reduced the incidence of eyespot without necessarily increasing yield. Glynne & Moore (1949) observed in field crops in Britain that the percentage straws severely affected by eyespot and the extent of lodging were less in thin than in dense crops; reducing the seed rate in pots also reduced the percentage severely diseased straws and tended to increase yield per pot of infected plants but not of controls.

(4) *Depth of sowing*. Rispens (1935), in Holland, stated that shallow-sown plants were more vigorous, had flatter tillers and seemed to suffer less from eyespot than deeply sown ones, possibly because they sloughed off infected leaves in dry weather. Field observations at Rothamsted gave some support to this view but pot experiments did not, suggesting that factors may operate in opposite ways in the two environments.

THE PRESENT EXPERIMENT

The present experiment was carried out on the northern half of Little Knott field, Rothamsted, on land put down to pasture in 1925 and ploughed up in 1942. Winter wheat was sown each autumn for six consecutive years from 1942. In the three years, 1943–5 respectively, 0, 63 and 93% of the straws were infected by eyespot at harvest, 0, 25 and 70% having severe eyespot lesions and 0, 10 and 70% of the area was lodged. The experiment was begun in the autumn of 1945 with fifty-four plots, each $\frac{1}{66}$th acre, consisting of two drill widths which included twenty-six rows 6 in.

apart. Plots were separated by paths 2–3 links wide, blocks by headlands 10 links wide running parallel with plots and 35-link headlands at right angles to the plots.

Treatments

(1) 12½% B.O.V. (by vol.) was sprayed from a horse-drawn machine, using 100 gal./acre. The treatment was applied both after sowing, before the wheat plants emerged on 20 Oct. 1945, 30 Oct. 1946 and 1 Nov. 1947 and again in spring on 16 Mar., 10 Apr. and 8 Mar. in three successive years. Snow lying late in 1947 delayed spraying till April when the wheat was in about the same stage as in March in the other two years.

(2) N_0, N_1, N_2 received respectively 0, 2 and 4 cwt./acre sulphate of ammonia on 21 Mar. 1946, 5 Apr. 1947 and 15 Mar. 1948.

(3) R_1, R_2, R_3 had 1½, 2½ and 3½ bushels of seed/acre except in the autumn of 1946 for the 1947 harvest when 1, 2 and 3 bushels/acre were sown. Seed of Squarehead's Master 13/4, treated with Ceresan, was sown on 15–16, 21 and 22 Oct. in the three successive years.

(4) The prescribed depths D_1, D_2, D_3 respectively were ½, 1½ and 3 in., but the heavy stony land at Rothamsted made it difficult to control depth of seeding.

Treatments (2), (3) and (4), each at three levels, provided twenty-seven treatment combinations which were arranged in a $3 \times 3 \times 3$ design in three blocks of nine plots. The three blocks were duplicated and one randomly selected from each pair containing similar treatments was sprayed with H_2SO_4. Blocks of nine were sprayed instead of single plots, because this reduced risk of re-infection by spores blown in rain, although it involved loss of precision in determining the effects of spraying.

The same treatments were applied each year to the same plots so that treatment effects were cumulative.

All plots received dressings of 3 cwt. superphosphate and 1 cwt. muriate of potash applied on 24 Oct. 1947 and 8 Oct. 1948; they were sprayed with DENOC on 14 May 1948 in an unsuccessful attempt to control weeds.

Sampling. All plots were sampled in March, May and July each year, except in 1947 when March sampling was prevented by snow. The number and area of samples removed is shown in Table 1. Each sample consisted of all plants in two parallel 6 or 12 in. rows. Pairs of wooden bars with metal rods protruding 6 and 12 in. apart greatly facilitated accurate sampling. When only two samples were taken per plot their positions were randomly determined; when four were taken they were treated as two sets of two; the first of each set was from a randomly selected point in one-half the plot, the second at a distance of one-half the length along and of the width across the plot avoiding the part within 1 ft. of the edge. Similarly, when six samples were taken they were treated as two sets of three; the first of each set was from a randomly selected point in one-third, the others at a distance of one-third the length down and the width across the plot from the previous one. Distribution of samples over the plots was thus ensured.

Recording. Plants taken in sampling were washed and records made in March and May of the numbers free from and of those infected by eyespot, sharp eyespot, take-all, brown footrot and other fungal diseases and some insect pests; the number of shoots free from and of those infected by eyespot and sharp eyespot was also recorded. In July similar records were made of straws, eyespot lesions being graded according to whether they were slight or severe.

The percentage areas lodged, those covered by weeds and those showing dwarfing and premature ripening, due to take-all, were estimated at intervals from the end of June till harvest each year. In 1948 green weight of weeds from 1 sq.yd. of six representative plots were determined.

TABLE 1. *Number of samples removed and area covered by samples*

Date of sampling	No. of samples per plot	Length in inches of two parallel rows in each sample	Total in fifty-four plots			
			Area sampled in sq.ft.	No. of plants	No. of shoots	No. of straws
15–21 Mar. 1946	2	6	54	1,414	2,444	—
22 May 1946	4	6	108	3,111	6,420	—
19–24 July 1946	6	12	324	—	—	9,467
14–15 May 1947	4	6	108	1,569	6,455	—
10–11 July 1947	6	12	324	—	—	7,120
2– 4 Mar. 1948	4	6	108	2,438	5,542	—
12–14 May 1948	4	6	108	2,481	9,168	—
23–29 July 1948	6	12	324	—	—	9,048
Total	—	—	1,458	11,013	30,029	25,635

The crops were harvested and yields of straw and grain determined by the farm staff. In 1946 all plants were taken from $\frac{1}{2}$ sq.yd. in each plot shortly before harvest; ears were counted and grain threshed in a small electric laboratory thresher. Samples of grain from the farm and laboratory samples were separated into dressed grain and tail corn by means of a sieve with meshes $2{\cdot}26 \times 20$ mm.; weed seeds harvested with the grain were counted. In December 1945 numbers of plants were counted in five randomly selected 1 ft. rows in each plot; in 1946 the numbers of ears emerged in four $2\frac{1}{2}$ ft. rows in each plot were counted at intervals in June and July.

Treatment of data. Most of the data were analysed by the Statistical Department. Certain 3-factor interactions were confounded with block differences. Results expressed as percentages were transformed using the angular transformation, to determine standard errors. Control and sprayed blocks were analysed separately; they are recorded separately when their results are dissimilar and the means given when they are alike.

Statistical analyses showed few significant effects of sowing at different depths; measurements of the distance between seed and green parts made on 244 plants in March 1946 and on 2027 plants in March 1948 gave as mean measurements in

inches of depths of seed for D_1, D_2 and D_3: 1·14, 1·07 and 1·91 in 1946, and 1·27, 1·43 and 1·62 in 1948, the differences achieved thus being much less than those attempted. The three treatments D_1, D_2 and D_3 have therefore been used as replicates for the other treatments.

The year in which it was harvested is used in referring to each crop.

Effects on plants and shoots

Plant density was highest in 1946 when 1½ bushels of seed/acre produced 10·0 plants/ft. row by May; it was lowest, after an exceptionally long cold winter in 1947 when 3 bushels of seed produced only 10·2 plants/ft. row. (The slight increase in plant number recorded in succeeding counts in the same crop may have been due to late germination of a few seeds, breaking of tillered plants or to sampling error.) The only treatment which produced a consistently significant effect was seed rate, the increase in plant number being less than proportional to that of seed rate, shown in Table 2.

Table 2. *Effect of seed rate on number of plants*

	Seed rate in bushels/acre for harvest years		Mean number of plants per foot of single row					
			1945	1946		1947	1948	
	1947	1946, 1948	Dec.	March	May	May	March	May
R_1	1	1½	8·2	9·0	10·0	4·6	8·4	8·2
R_2	2	2½	12·4	13·0	15·0	7·0	11·6	11·8
R_3	3	3½	17·3	17·3	18·2	10·2	13·8	14·4
Mean			12·6	13·1	14·4	7·3	11·3	11·5
Standard errors			—	—	±0·54	±0·46	—	±0·60
Standard errors of means			—	—	±0·31	±0·27	—	±0·35

Table 3. *Effect of sulphate of ammonia and of seed rate on number of shoots per plant*

	1946			1947	1948		
Treatment	March	May	Decrease between March and May	May	March	May	Increase between March and May
N_0	3·64	2·16	1·48	3·82	2·29	3·79	1·50
N_1	3·49	2·20	1·29	4·54	2·16	3·90	1·74
N_2	3·48	2·24	1·24	5·00	2·44	4·14	1·70
R_1	3·78	2·79	0·99	5·37	2·42	4·93	2·51
R_2	3·55	2·06	1·49	4·48	2·26	3·62	1·36
R_3	3·27	1·76	1·51	3·52	2·22	3·29	1·07
Mean	3·53	2·20	1·33	4·46	2·30	3·95	1·65

Maximum shoot number was probably reached between March and May each year. It was greater in March than in May in 1946, less in 1948 (Table 3). Sulphate of ammonia increased the number of shoots per plant by May each year, by reducing death of shoots in 1946 and by greater increase in their number in 1948. The number of shoots per plant was highest in 1947 when plant number was lowest, and each year

decreased with increase in plant number and so with seed rate. The effect of seed rate was evident in March, increasing by May, in 1946 by more death and in 1948 by less increase of shoot number in the densely than in the thinly sown plots.

Number of shoots per foot row

Like the number of shoots per plant, the number per foot decreased between March and May in 1946 and increased in 1948 (Table 4). Sulphate of ammonia tended to increase the number of shoots per foot in May. Increase in seed rate each year increased the number of shoots per foot, the effect being greater in March than in May.

TABLE 4. *Effect of sulphate of ammonia and of seed rate on number of shoots per foot of row*

	1946		1947	1948	
Treatment	March	May	May	March	May
N_0	50·2	28·1	27·6	25·4	41·8
N_1	42·4	30·0	30·8	23·9	40·5
N_2	43·2	30·7	31·2	27·7	45·0
R_1	34·0	26·7	24·1	20·0	39·5
R_2	45·2	30·6	30·8	26·1	42·2
R_3	56·5	31·6	34·8	30·9	45·6
Mean	45·3	29·6	29·9	25·7	42·4
Standard errors	—	±1·01	±1·55	—	±1·28
Standard errors of means	—	±0·58	±0·90	—	±0·74

TABLE 5. *Effect of sulphate of ammonia and of seed rate on number of straws in July*

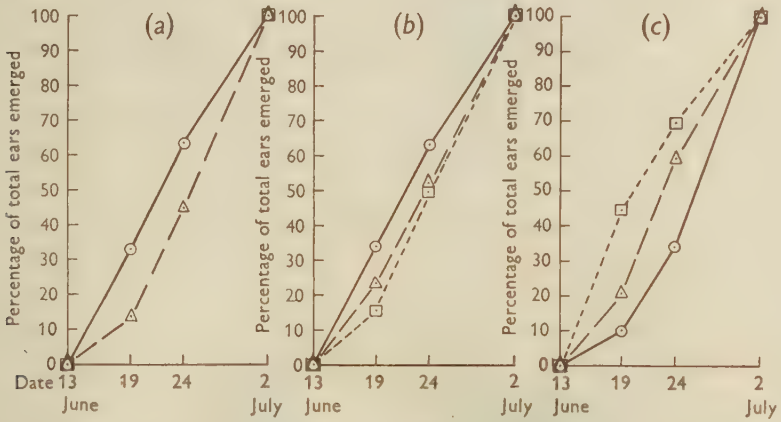
	1946		1947		1948	
Treatment	Control	Sprayed	Control	Sprayed	Control	Sprayed
N_0	14·2	13·7	10·2	10·1	13·7	13·9
N_1	14·8	14·6	10·9	11·6	11·5	15·9
N_2	15·4	14·9	11·6	11·6	11·8	15·8
R_1	13·6	13·6	10·1	9·8	11·6	14·7
R_2	15·5	15·0	10·9	11·3	12·3	16·4
R_3	15·4	14·5	11·6	12·2	13·3	14·7
Mean	14·8	14·4	10·9	11·1	12·4	15·2
Standard errors	±0·61		±0·45		±0·76	
Standard errors of means	±0·35		±0·26		±0·44	

Number of straws in July

The treatments had much less effect on number of straws than on number of shoots (Table 5). Straw numbers were but little affected by spraying except in control blocks in 1948 when they were increased by 23%. They were increased by sulphate of ammonia except in control blocks in 1948; they were generally increased by seed rate R_1 being less than the mean of R_2R_3.

Number of ears

Ears were counted both in the standing crop and in ½ yd.sq. samples in 1946 but not in other years. Rather higher figures were obtained in the standing crop (probably because it was difficult to exclude all outlying ears). Both counts showed small increases in number of ears when sulphate of ammonia was applied and when seed rate was increased. There were rather fewer ears in sprayed than in control plots. In the standing crop on 2 July means for control and sprayed were 18·2 and 16·8; for N_0, N_1, N_2, 16·4, 17·8 and 18·4; for R_1, R_2, R_3, 16·5, 17·9 and 18·2 respectively.



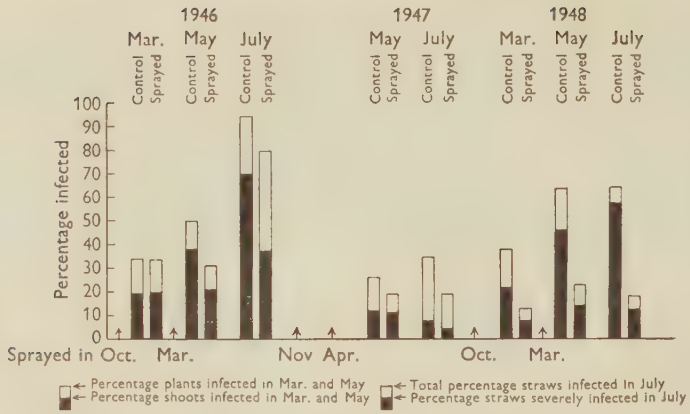
Text-fig. 1. 1946. Date of ear emergence. (*a*) Effect of spraying. ⊙, control; △, sprayed. (*b*) Effect of sulphate of ammonia. ⊙, N_0, 0 cwt./acre; △, N_1, 2 cwt./acre; ⊡, N_2, 4 cwt./acre. (*c*) Effect of seed rate. ⊙, R_1, 1½ bushels/acre; △, R_2, 2½ bushels/acre; ⊡, R_3, 3½ bushels/acre.

The effect of each treatment on rate of ear emergence was determined by converting the number emerged at different dates into percentage of the number appearing under each treatment by 2 July, when practically all had emerged. The spaces between lines shown in Text-fig. 1 *a–c* show the delay in emergence resulting from each treatment. Spraying delayed emergence by 3–4 days; 4 cwt. sulphate of ammonia had about the same effect; each reduction of 1 bushel of seed delayed emergence by 3–4 days.

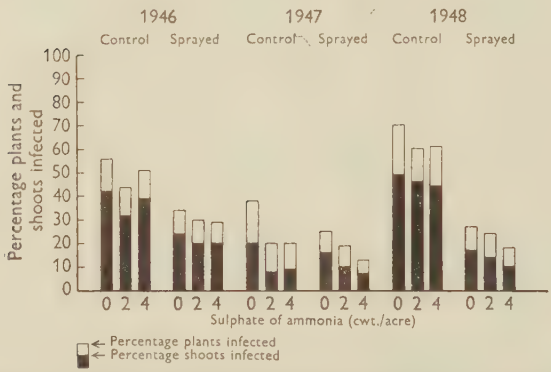
Disease incidence

Eyespot (*Cercosporella herpotrichoides*). In control plots eyespot was prevalent in the wet seasons of 1946 and 1948 but not after the cold winter and late spring of 1947.

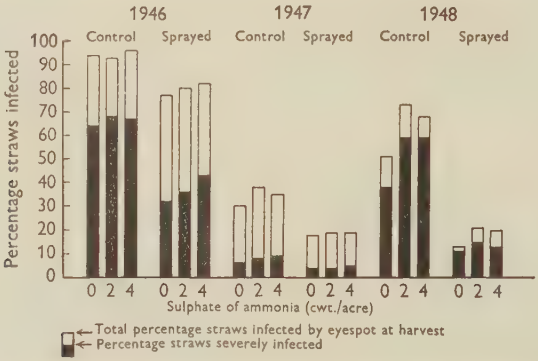
Spraying the previous autumn showed no effect in March in 1946, but the cumulative effect of previous autumn and spring spraying had greatly reduced eyespot incidence by March 1948. Percentage plants and shoots infected in May, and straws infected in July, were consistently less in sprayed than in control blocks each year, the difference between control and sprayed being greatest in 1948, least in 1947.



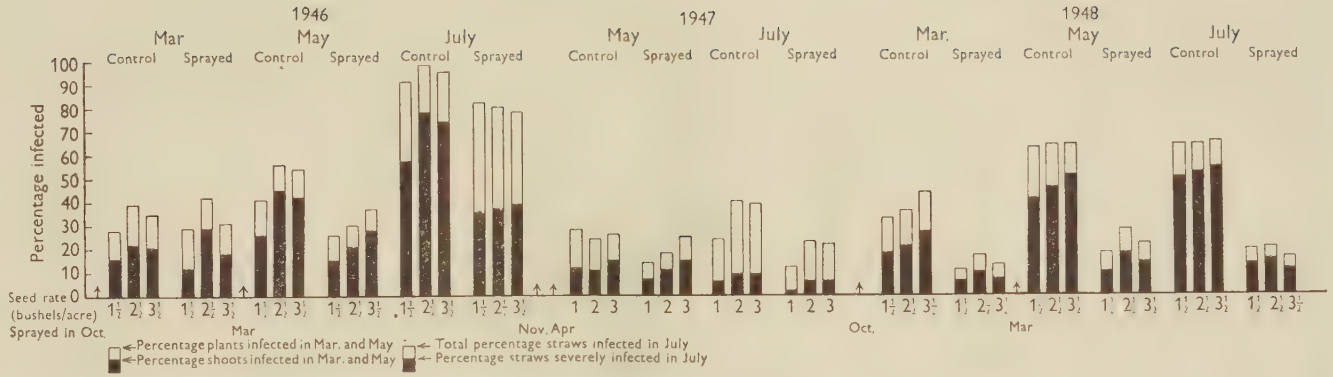
Text-fig. 2. Effect of spraying on eyespot incidence.



Text-fig. 3*a*. Effect of sulphate of ammonia applied in spring on eyespot incidence in May.



Text-fig. 3*b*. Effect of sulphate of ammonia applied in spring on eyespot incidence in July.



Text-fig. 4. Effect of seed rate on incidence of eyespot.

TABLE 6. *Effect of sulphate of ammonia and of seed rate on eyespot incidence in July*

Harvest years	Seed rate (bushels/acre)	Control Sulphate of ammonia (cwt./acre) 0	2	4	Mean	Sprayed Sulphate of ammonia (cwt./acre) 0	2	4	Mean	Effect spraying
		Total percentage straws infected by eyespot								
1946	1½	92·0	84·6	95·7	90·8	81·7	79·2	83·9	81·6	− 9·2
	2½	96·8	97·8	98·6	97·7	72·0	81·0	87·2	80·1	−17·6
	3½	92·1	97·9	94·4	94·8	78·3	81·3	73·3	77·6	−17·2
	Mean	93·6	93·4	96·2	94·4	77·3	80·5	81·5	79·8	−14·6
	Effect spraying					−16·3	−12·9	−14·7	−14·6	
1947	1	22·9	25·6	23·0	23·8	10·8	13·5	12·4	12·2	−11·6
	2	32·7	43·7	45·3	40·6	17·4	24·9	25·8	22·7	−17·9
	3	35·7	44·8	37·6	39·4	25·3	20·9	19·7	22·0	−17·4
	Mean	30·4	38·0	35·3	34·6	17·8	19·8	19·3	19·0	−15·6
	Effect spraying					−12·6	−18·2	−16·0	−15·6	
1948	1½	55·0	76·3	60·7	64·0	12·7	21·7	21·7	18·7	−45·3
	2½	52·3	65·7	74·3	64·1	17·7	22·0	20·7	20·1	−44·0
	3½	46·0	79·0	68·7	64·6	9·7	20·7	17·0	15·8	−48·8
	Mean	51·1	73·7	67·9	64·2	13·4	21·5	19·8	18·2	−46·0
	Effect spraying					−37·7	−52·2	−48·1	−46·0	
		Percentage straws severely infected by eyespot								
1946	1½	52·6	44·5	74·8 (1)	57·3 (2)	31·1	31·2	45·7 (1)	36·0 (2)	−21·3
	2½	76·9	79·1	79·5	78·5	32·1	34·5	44·9	37·2	−41·3
	3½	64·1	80·7	77·4	74·1	33·8	43·0	39·2	38·7	−35·4
	Mean	64·5	68·1	77·2 (2)	70·0	32·3	36·2	43·3 (2)	37·3	−32·7
	Effect spraying					−32·2	−31·9	−33·9	−32·7	
1947	1	6·7	5·8	4·7 (1)	5·7 (2)	0·9	1·9	2·8 (1)	1·9 (2)	− 3·8
	2	4·0	9·3	12·4	8·6	5·8	4·3	6·5	5·5	− 3·1
	3	7·3	10·4	9·9	9·2	6·2	6·3	5·0	5·8	− 3·4
	Mean	6·0	8·5	9·0 (2)	7·8	4·3	4·2	4·8 (2)	4·4	− 3·4
	Effect spraying					−1·7	−4·3	−4·2	−3·4	
1948	1½	41·7	56·0	51·7 (1)	49·8 (2)	10·3	14·0	13·3 (1)	12·6 (2)	−37·2
	2½	38·3	52·7	64·3	51·8	14·7	15·7	13·7	14·7	−37·1
	3½	32·7	68·0	60·7	53·8	7·0	14·3	11·7	11·0	−42·8
	Mean	37·6	58·9	58·9 (2)	51·8	10·7	14·7	12·9 (2)	12·8	−39·0
	Effect spraying					−26·9	−44·2	−46·0	−39·0	

Standard errors of angular transformation of percentage severe eyespot at harvest:

	1946	1947	1948 Control	1948 Sprayed
Means of 3 plots, e.g. (1)	±4·71	±3·08	±3·74	±1·48
Means of 9 plots, e.g. (2)	±2·72	±1·78	±2·16	±0·86
Effect spraying (for comparison main effects only, i.e. means 9 plots)	±3·85	±2·52	—	±2·32

(Standard errors of control and sprayed were different enough to be quoted separately only in 1948.)

Sulphate of ammonia supplied after sampling in March, generally reduced the percentage plants and shoots infected with eyespot in May and increased percentage straws severely infected in July.

Increase in seed rate tended to increase percentage of plants and shoots infected by eyespot in March and May, and of straws infected in July, except on sprayed plots in 1948 when take-all reduced the number of straws per foot in the most densely sown plots (Table 6).

TABLE 7. *Development of take-all*, Ophiobolus graminis

A. Percentage straws infected by take-all in July

Harvest years	Seed rate (bushels/acre)	Mean control and sprayed N_0	N_1	N_2	Mean
1947	1	1·0	0·7	0	0·6
	2	1·2	0	0·5	0·6
	3	0·8	0·6	0	0·5
	Mean	1·0	0·4	0·2	0·5
1948	1½	34·0	23·0	8·6 (1)	21·9 (2)
	2½	33·0	24·0	22·0	26·3
	3½	57·2	18·8	16·5	30·8
	Mean	41·4	22·0	15·7 (2)	26·4

Standard errors of angular transformations of percentage straws infected by take-all in July 1948:

Means of 6 plots, e.g. (1) ±3·54
Means of 18 plots, e.g. (2) ±2·04

B. Percentage area covered by take-all patches in 1948

Date	Seed rate (bushels/acre)	Mean control and sprayed N_0	N_1	N_2	Mean
30 June	1½	5·2	2·2	0	2·5
	2½	7·5	0·4	1·2	3·0
	3½	19·8	1·8	0·8	7·5
	Mean	10·8	1·5	0·7	4·3
14 July	1½	9·5	4·8	0·6	5·0
	2½	16·6	4·2	2·6	7·8
	3½	41·4	4·2	1·7	15·8
	Mean	22·5	4·4	1·6	9·5
26 July	1½	20·0	12·6	2·2	11·6
	2½	30·2	10·5	5·4	15·4
	3½	65·2	18·0	9·0	30·7
	Mean	38·5	13·7	5·5	19·2
10 Aug.	1½	38·0	32·8	7·7	26·2
	2½	42·8	20·5	13·5	25·6
	3½	82·5	30·5	18·8	43·9
	Mean	54·4	27·9	13·3	31·9

Take-all (*Ophiobolus graminis*) was found on only a few plants in 1946. In July 1947 the disease occurred in all blocks, but not more than 4% plants were found infected in any plot. In 1948 more than half the plants in some plots were severely infected by take-all at harvest (Table 7). Patches of plants dwarfed and ripening

early appeared towards the end of June and increased in area till harvest; whiteheads occurred mostly in these patches about the end of July, turning black in August because invaded by saprophytic fungi. Spraying had no apparent effect on take-all. Both percentage straws infected in July and areas covered by take-all patches were reduced by sulphate of ammonia and were increased by increasing seed rate.

Sharp eyespot (*Corticium* (*Rhizoctonia*) *solani*). There was no evidence of appreciable damage to the crop caused by sharp eyespot. The disease occurred each year, tending to be most common when eyespot was least. Treatments showed significant effects only in 1947 when sulphate of ammonia increased and heavier seed rate decreased the percentage infected in control plots (Table 8).

TABLE 8. *Incidence of sharp eyespot*

	Percentage infected by sharp eyespot				
	March		May		July
Harvest year	Plants	Shoots	Plants	Shoots	Straws
1946	5	2	16	10	3
1947	—	—	7	3	20
1948	5	2	8	4	16

TABLE 9. *Effect of sulphate of ammonia on brown footrot*, Fusarium *sp.*

		No. infected		
Date	No. examined	N_0	N_1	N_2
May 1946	3111 plants	0	4	10
July 1947	7120 straws	34	135	165
July 1948	9048 straws	7	29	39

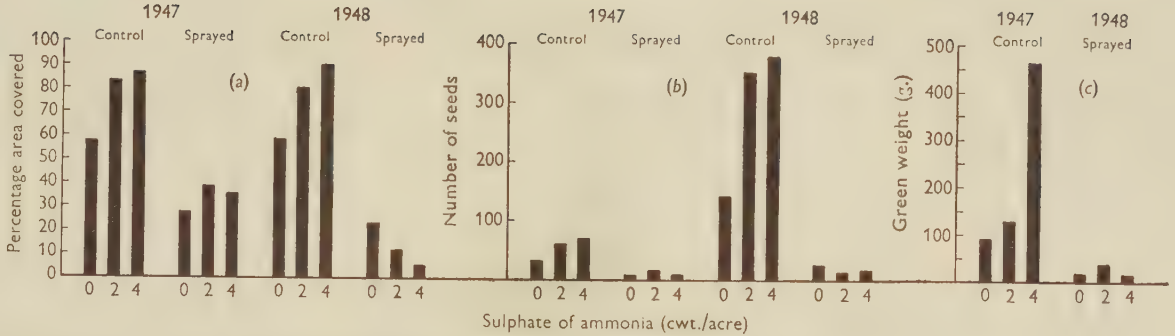
Brown footrot (*Fusarium* sp.) was never severe, affecting less than 1 % of the straws except in the hot summer of 1947, when nearly 5 % were infected (Table 9); the disease increased with each application of sulphate of ammonia. A similar increase in the frequency of brown footrot in hot summers and with application of sulphate of ammonia had been noted in previous years on Broadbalk field.

Other diseases and pests. Leaf spot, *Septoria tritici*, was noted occasionally in March; wheat bulb fly, *Leptohylemia coarctata*, in March and May and Hessian fly, *Mayetiola destructor*, in July.

WEEDS

Weeds were few in 1946, abundant in the two following years. In 1947 mayweed, *Matricaria chamomilla*, predominated, speedwell, *Veronica hederifolia*, and goosegrass, *Gallium aparine*, were abundant. In 1948 there was hardly any mayweed; speedwell predominated early in the season and was later superseded by goosegrass. Although similar areas were covered by weeds in both years, many more weed seeds were harvested with the grain in 1948, when goosegrass, with seeds large enough to be retained in threshing, was dominant.

Spraying consistently reduced the area covered by weeds (Text-fig. 5), the green



Text-fig. 5. Effect of spraying with H_2SO_4 and of sulphate of ammonia applied in spring, on weeds at end of July. (*a*) Percentage area covered by weeds. (*b*) Number weed seeds harvested with 100 g. grain. (*c*) Green weight of weeds in g. per sq.yd. (two samples $\frac{1}{2}$ sq.yd. for each treatment).

weight of weeds and the number of weed seeds harvested with the grain, but thistles, *Carduus arvensis*, and dock, *Rumex* sp., survived after spraying.

Sulphate of ammonia increased weeds in control plots; it had less effect on sprayed plots, but in 1948 it reduced the area covered by weeds probably because the plots low in nitrogen had patches of plants dwarfed by take-all and these patches were easily colonized by weeds. The area covered by weeds and the number of weed seeds harvested with grain decreased with increasing seed rate.

TABLE 10. *Yield of straw (cwt./acre)*

Harvest year		Control N_0	N_1	N_2	Mean		Sprayed N_0	N_1	N_2	Mean		Effect spraying
1946	R_1	66·1	65·6	70·4 (1)	67·4 (2)	R_1	66·5	70·1	70·1 (1)	68·9 (2)		+1·5
	R_2	60·7	71·9	68·7	67·1	R_2	61·9	67·7	71·5	67·0		−0·1
	R_3	57·6	68·9	73·2	66·6	R_3	61·5	71·6	71·5	68·2		+1·6
Mean		61·5	68·8	70·8 (2)	67·0		63·3	69·8	71·0 (2)	68·0	67·5	
Effect spraying							+1·8	+1·0	+0·2	+1·0		
1947	R_1	32·7	35·8	41·4 (1)	36·6 (2)	R_1	31·0	35·9	38·1 (1)	35·0 (2)		−1·6
	R_2	34·5	38·8	39·5	37·6	R_2	30·1	36·2	40·6	35·6		−1·9
	R_3	29·9	37·6	44·5	37·3	R_3	31·5	40·8	41·2	37·9		+0·6
Mean		32·4	37·4	41·7 (2)	37·2		30·9	37·7	40·0 (2)	36·2	36·7	
Effect spraying							−1·5	+0·3	−1·7	−1·0		
1948	R_1	56·7	51·7	62·7 (1)	57·0 (2)	R_1	64·3	79·2	88·8 (1)	77·4 (2)		+20·4
	R_2	57·0	59·3	68·3	61·5	R_2	61·1	76·2	79·7	72·3		+10·8
	R_3	42·9	56·2	56·9	52·0	R_3	57·9	77·9	85·1	73·6		+21·6
Mean		52·2	55·7	62·6 (2)	56·9		61·1	77·8	84·5 (2)	74·5	65·7	
Effect spraying							+8·9	+22·1	+21·9	+17·6		

	Standard errors 1946	1947	1948
Means for 3 plots, e.g. (1)	±2·89	±2·21	±3·13
Means for 9 plots, e.g. (2)	±1·69	±1·28	±1·81
Effect spraying (for comparison main effects only, i.e. means 9 plots)	±2·36	±1·81	±2·45

STRAW

Straw yield was exceptionally low in 1947, when it was only about 55% of that obtained in other years (Table 10).

Spraying had little effect, except in 1948, when it increased mean yield by 8·9 cwt./acre on plots which received no nitrogen and by 22·0 cwt./acre on those to which sulphate of ammonia was applied.

Each dose of sulphate of ammonia increased weight of straw: in 1948 its effect on sprayed plots was more than twice that in other years because it reduced take-all; but on unsprayed plots the increase in yield caused by decreasing take-all seems to have been counterbalanced by the reduction in yield due to heavier and earlier infection by eyespot.

Increase in seed rate generally decreased yield of straw on plots which had no sulphate of ammonia, the effect being greatest in 1948 when take-all was severe. But

on plots which received sulphate of ammonia seed rate had little effect, suggesting that the more fertile plots were better able to support a denser population.

Height was measured only in the last two years, the mean for 1947 being 41·7 in. and for 1948, 48·0 in. Spraying had no effect in 1947 but increased mean height by 3·5 in. in 1948. Sulphate of ammonia slightly increased height in 1947, the means for N_2 being 1·4 and 2·1 in. higher than for N_0 in control and sprayed blocks respectively; in 1948 it had no effect on control but on sprayed plots the mean for N_2 was 4·3 in. higher than for N_0.

Rate of sowing had little effect except on control plots in 1948 when the mean for R_3 was 2·6 in. shorter than that for R_1.

TABLE 11. *Percentage area lodged*

Harvest years	Date	Seed rate (bushels/acre)	Control				Sprayed				Effect spraying
			N_0	N_1	N_2	Mean	N_0	N_1	N_2	Mean	
1946	19 July	1½	60	27	77	55	0	3	7	3	−52
		2½	82	91	92	88	0	9	27	12	−76
		3½	48	91	88	76	3	29	46	26	−50
		Mean	63	70	86	73	1	14	27	14	
		Effect spraying					−62	−56	−59	−59	
	21 Aug.	1½	91	74	91	86	11	5	35	17	−69
		2½	97	97	96	97	12	28	40	27	−70
		3½	74	98	95	89	21	58	75	51	−38
		Mean	88	90	94	90	15	30	50	31	
		Effect spraying					−73	−60	−44	−59	
1948	14 July	1½	1	3	25	10	0	0	0	0	−10
		2½	2	17	34	18	0	0	0	0	−18
		3½	0	9	30	13	0	0	0	0	−13
		Mean	1	10	30	14	0	0	0	0	
		Effect spraying					−1	−10	−30	−14	
	16 Aug.	1½	18	53	84	52	0	0	1	0	−52
		2½	20	83	95	66	1	1	8	3	−63
		3½	7	70	87	55	0	3	3	2	−53
		Mean	15	69	89	58	0	1	4	2	
		Effect spraying					−15	−68	−85	−56	

LODGING

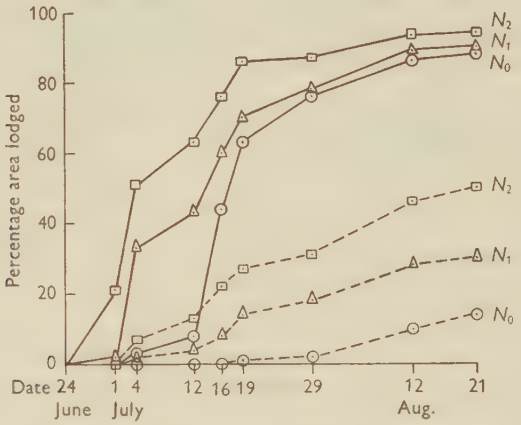
In 1946 lodging began at the end of June, was accelerated by thunderstorms on 3, 17 and 26 July, and at harvest 90% of control and 31% of sprayed blocks were flat. There was no lodging in 1947; in 1948 it began at the end of June, and at harvest 58% of control and only 2% of the sprayed blocks were laid. Table 11 shows the percentage areas lodged both at harvest and 33 days earlier, in 1946 and 1948.

Spraying had a great effect, delaying lodging and reducing the percentage area lodged at harvest by similar amounts, 59 and 56% in the two years.

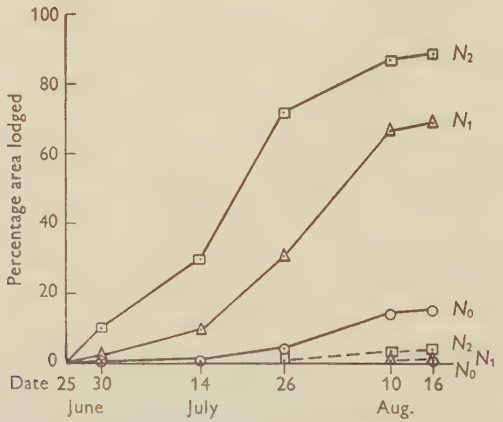
Each increment of sulphate of ammonia increased weight of straw and the percentage severely infected by eyespot, both effects increasing the area lodged in 1946 (Pl. 20, figs. 1–3). In 1948 take-all caused stunting which reduced lodging most on

the nitrogen-deficient plots, thus increasing the effect of the fertilizer on lodging (Text-figs. 6, 7, 8).

Increase in seed rate increased lodging in 1946 when 1 bushel of seed had about as much effect as 2 cwt./acre sulphate of ammonia (Pl. 20, figs. 4–6). In 1948 take-all



Text-fig. 6. 1946. Effect of sulphate of ammonia applied in spring on percentage area lodged. ⊙, N_0, 0 cwt./acre; △, N_1, 2 cwt./acre; ⊡, N_2, 4 cwt./acre. ——, control; - - - -, sprayed.



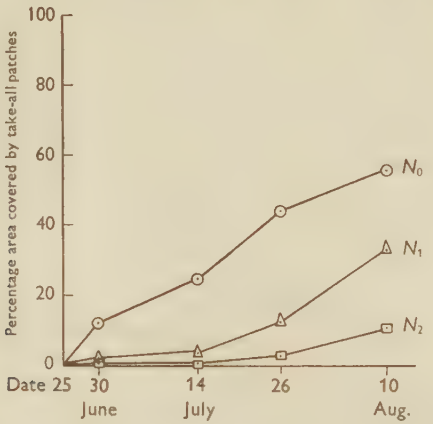
Text-fig. 7. 1948. Effect of sulphate of ammonia applied in spring on percentage area lodged. △, N_0, 0 cwt./acre; △, N_1, 2 cwt./acre; ⊡, N_2, 4 cwt./acre. ——, control; - - - -, sprayed.

reduced lodging most in the densely sown plots thus reducing the effect of seed rate of lodging (Text-figs. 9, 10, 11).

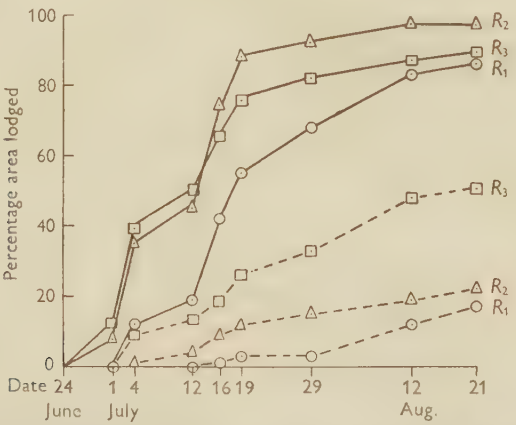
The percentage area lodged depended partly on the weight of straw, partly on the percentage straws with severe eyespot lesions. Table 12 includes the records of the

fifty-four plots in each of three years giving 162 records for percentage area lodged both at harvest and 33 days earlier.

To calculate partial regressions only groups of plots in which considerable lodging occurred could be used. This reduced the number to 81 (control and sprayed in 1946



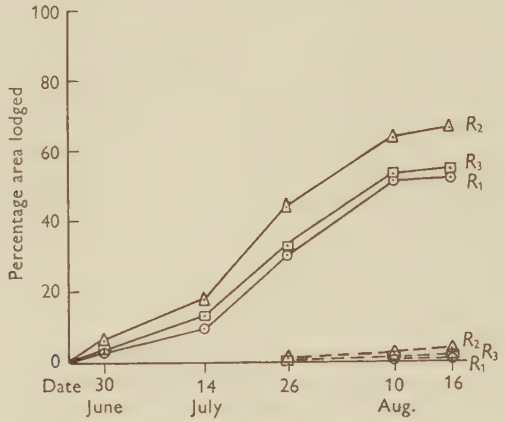
Text-fig. 8. 1948. Effect of sulphate of ammonia applied in spring on percentage area covered by take-all patches in control plots. ⊙, N_0, 0 cwt./acre; △, N_1, 2 cwt./acre; ⊡, N_2, 4 cwt./acre.



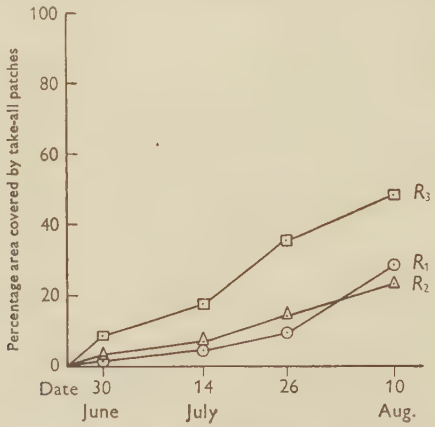
Text-fig. 9. 1946. Effect of seed rate on percentage area lodged. ·, R_1, 1½ bushels/acre; △, R_2, 2½ bushels/acre; ⊡, R_3, 3½ bushels/acre. ——, control; - - - -, sprayed.

and control in 1948). The partial regression coefficient of percentage area lodged at harvest on weight of straw (eliminating eyespot) was $1{\cdot}106 \pm 0{\cdot}239$ and on eyespot (eliminating straw) was $0{\cdot}781 \pm 0{\cdot}138$; that of percentage area lodged 33 days before harvest was $0{\cdot}968 \pm 0{\cdot}197$ on weight of straw and $0{\cdot}925 \pm 0{\cdot}114$ on eyespot, showing

that both weight of straw and percentage severely infected by eyespot had highly significant effects on lodging; the regressions accounted for 51% of the variance in percentage area lodged at harvest and 64% of that occurring 33 days earlier.



Text-fig. 10. 1948. Effect of seed rate on percentage area lodged. ⊙, R_1, 1½ bushels/acre; △, R_2, 2½ bushels/acre; ⊡, R_3, 3½ bushels/acre. ——, control; - - - -, sprayed.



Text-fig. 11. 1948. Effect of seed rate on percentage area covered by take-all patches in control plots. ⊙, 1½ bushels/acre; △, 2½ bushels/acre; ⊡, 3½ bushels/acre.

YIELD OF GRAIN

Table 13 shows that in the three successive years, 1946, 1947 and 1948 mean grain yields fell from 27·0 to 26·0 to 19·4 cwt./acre, the low yield in 1948 being partly due to the prevalence of take-all. Yields on the most thickly sown control plots with no nitrogen fell from 26·0 to 22·5 to 11·7 cwt./acre (means for three plots D_1, D_2 and D_3)

TABLE 12. *Effect of weight of straw and of percentage straws severely infected by eyespot on percentage area lodged*

	Mean percentage area lodged — Percentage straws severely infected by eyespot											
	0–20			21–50			51–70			71–100		
		% area lodged			% area lodged			% area lodged			% area lodged	
Weight straw (cwt./acre)	No. plots	At harvest, A	33 days before harvest, B	No. plots	At harvest, A	33 days before harvest, B	No. plots	At harvest, A	33 days before harvest, B	No. plots	At harvest, A	33 days before harvest, B
Over 25, up to 40	39	0	0	0	—	—	1	40	0	0	—	—
Over 40, up to 60	20	0	0	13	30	3	6	62	20	5	95	66
Over 60, up to 95	24	7	2	23	34	11	16	78	43	15	94	81

TABLE 13. *Yield of grain (cwt./acre)*

Harvest years		Control N_0	Control N_1	Control N_2	Control Mean		Sprayed N_0	Sprayed N_1	Sprayed N_2	Sprayed Mean	Mean control and sprayed	Effect spraying
1946	R_1	26·6	26·8	25·0 (1)	26·1 (2)	R_1	26·4	28·1	28·4 (1)	27·6 (2)		+1·5
	R_2	27·0	26·1	23·9	25·7	R_2	26·8	29·1	28·5	28·1		+2·5
	R_3	26·0	26·6	26·6	26·4	R_3	26·3	29·3	28·0	27·9		+1·5
	Mean	26·5	26·5	25·2 (2)	26·1		26·5	28·8	28·3 (2)	27·9	27·0	
	Effect spraying						0	+2·3	+3·1	+1·8		
1947	R_1	21·5	23·2	26·7 (1)	23·8 (2)	R_1	23·0	27·0	27·0 (1)	25·7 (2)		+1·9
	R_2	24·5	26·2	26·8	25·8	R_2	23·5	27·6	29·9	27·0		+1·2
	R_3	22·5	26·1	29·2	26·0	R_3	23·6	29·2	30·3	27·7		+1·8
	Mean	22·8	25·1	27·6 (2)	25·2		23·4	28·0	29·1 (2)	26·8	26·0	
	Effect spraying						+0·5	+2·8	+1·5	+1·6		
1948	R_1	17·7	13·6	17·3 (1)	16·2 (2)	R_1	18·9	26·2	29·1 (1)	24·7 (2)		+8·5
	R_2	17·0	16·1	17·6	16·9	R_2	19·1	23·5	24·3	22·3		+5·4
	R_3	11·7	16·1	14·7	14·2	R_3	15·0	24·7	27·2	22·3		+8·1
	Mean	15·5	15·2	16·5 (2)	15·8		17·7	24·8	26·9 (2)	23·1	19·4	
	Effect spraying						+2·2	+9·6	+10·4	+7·3		

	Standard errors		
	1946	1947	1948
Means for 3 plots, e.g. (1)	±1·13	±1·21	±1·17
Means for 9 plots, e.g. (2)	±0·65	±0·70	±0·67
Effect spraying (for comparison main effects only, i.e. means 9 plots)	±0·92	±0·99	±0·95

but yields of 28·4, 29·9 and 29·1 cwt./acre were maintained on sprayed plots sown at 1½–2 bushels/acre which received 4 cwt. of sulphate of ammonia.

Spraying increased mean yield of grain each year, the effect being greatest in 1948 least in 1947. Its effect on yield of plots receiving no nitrogenous fertilizer was negligible, but on those receiving sulphate of ammonia spraying increased mean yields by 2·7, 2·2 and 10·0 cwt./acre in the three successive years. It showed no consistent difference in effect at different seed rates.

Although sulphate of ammonia always increased straw, it increased grain only when lodging was controlled by spraying or by weather (Table 14). The effect of the fertilizer was greatest in 1948 on the sprayed plots (which had upright crops), because then it not only nourished the plants but also decreased take-all.

TABLE 14. *Effect of lodging and take-all on increments in grain yield for each dose of 2 cwt./acre sulphate of ammonia*

		1946		1947		1948	
		Control	Sprayed	Control	Sprayed	Control	Sprayed
Increment in grain yield (cwt./acre)	N_1–N_0	0	+2·3	+2·3	+4·6	−0·3	+7·1
	N_2–N_1	−1·3	−0·5	+2·5	+1·1	+1·3	+2·1
Percentage area lodged at harvest	N_0	88	15	0	0	15	0
	N_1	90	30	0	0	69	1
	N_2	94	50	0	0	89	4
Percentage area covered by take-all patches, 26 July	N_0	0	0	0	0	44·0	32·9
	N_1	0	0	0	0	12·2	15·2
	N_2	0	0	0	0	2·8	8·2

Seed rate showed no effect on grain yield in 1946 when the (mean for control and sprayed) number of plants per foot varied from 10·0 to 18·2. In 1947, 1 bushel of seed, producing only 4·5 plants/ft. yielded less than did 2 bushels which yielded about as much grain as 3 bushels of seed producing respectively 7·0 and 10·2 plants/ft. In 1948, when take-all was so severe on densely sown plots, 1½ bushels of seed producing 8·2 plants/ft. yielded about as much in control and more in sprayed plots than did higher rates.

Comparison with grain yield on Broadbalk. On the neighbouring Broadbalk field, where wheat has been harvested each year since 1844, plots 5–7 have, since 1852, received manurial treatments similar to plots receiving N_0, N_1 and N_2 in the present experiment. Seed rate varied on Broadbalk in the early years, but has been constant at 3 bushels/acre since 1928 when the new fallowing system was begun whereby one-fifth of every plot is now fallowed each year. Means of yields on plots sown at 2½ and 3½ bushels/acre in the present experiment are thus comparable with those now obtained on Broadbalk. By 1948, in the sixth successive wheat crop on Little Knott, yields had fallen to the level of those recorded on Broadbalk since 1852 (the ninth successive crop), and now obtained in the sections following a crop. In Table 15 the

increase in yield after a year's fallow on Broadbalk in 1948 is compared with that resulting from spraying on Little Knott.

Spraying and fallow had similar average effects in increasing yield, but fallow had its greatest effect on plots with least nitrogen, spraying on those with most nitrogen. Both treatments controlled weeds; they differed in that fallow increased available nitrogen and reduced take-all and so had most effect where nitrogen was deficient; spraying appeared to have no effect on available nitrogen or on take-all, but reduced eyespot and therefore lodging and so had most effect in the most fertile plots.

TABLE 15. 1948. *Effect on Broadbalk of 1 year's fallow compared with effect on Little Knott of spraying with* H_2SO_4

		Yield (cwt./acre)			Increment due to fallow (cwt./acre)			
	Treatment	Plot 5	Plot 6	Plot 7				
Field	Years after fallow	N_0	N_1	N_2	N_0	N_1	N_2	Mean
Broadbalk	2, 3, 4	11·7	16·4	22·8	—	—	—	5·9
	1	20·9	21·6	26·1	9·2	5·2	3·3	—
					Increment due to spraying			
					N_0	N_1	N_2	Mean
Little Knott	Control	14·4	16·1	16·2	—	—	—	6·8
	Sprayed	17·0	24·1	25·8	2·6	8·0	9·6	—

TABLE 16. *Effect of sulphate of ammonia on percentage tail corn*

Samples from ½ sq. yd. threshed in laboratory. Percentage tail corn (2nds and 3rds)

		N_0	N_1	N_2	Mean	
1946	Control	10·2	15·6	27·6	17·8	
	Sprayed	6·3	9·9	18·5	11·6	14·7
	Field samples from farm. Percentage tail corn					
1946	Control	5·1	9·5	11·3	8·6	
	Sprayed	5·3	6·3	7·9	6·5	7·6
1947	Control	3·8	3·7	4·2	3·9	
	Sprayed	4·4	4·8	5·7	5·0	4·4
1948	Control	24·1	26·8	27·9	26·3	
	Sprayed	29·2	22·9	27·6	26·6	26·4

The present experiment gives no means for determining the relative importance of each effect.

Tail corn. The small laboratory thresher gave a higher proportion of tail corn than the farm thresher in which the very light grain was probably blown away with the chaff. Table 16 shows that there was little tail corn in 1947 when eyespot, lodging and take-all were negligible, more in 1946 when eyespot and lodging were severe and very much more in 1948 when take-all was also severe. Spraying reduced the proportion of tail corn in 1946; sulphate of ammonia increased it each year; seed rate and depth of sowing had little effect.

The grain straw ratio was 0·40, 0·71 and 0·30 in the three successive years. Spraying increased it a little each year and sulphate of ammonia decreased it slightly except on sprayed plots in 1948.

DISCUSSION

Cumulative and weather effects

As similar treatments were applied to the same plots each year, cumulative effects cannot be distinguished with certainty from those depending on weather. But the sequence of annual fluctuations gives some indication of which influences are likely to have been most important.

The increase in eyespot incidence from 1943 to the high level attained in 1945 resulted as a cumulative effect of continuous wheat growing under weather conditions favourable to infection, but its subsequent fluctuations, being high in 1946, 1948 and 1949, and low in 1947, no doubt depended chiefly on weather conditions. Similar fluctuations in straw yield and in percentage areas lodged (which depended on eyespot and weight of straw) also depended on weather.

Take-all developed more slowly than eyespot, being noted first as slight in 1947, and severe in 1948 (and severe in 1949 after the experiment ended). This progressive rise was obviously a cumulative effect, probably increased by weather conditions; a mild preceding winter and very wet January made 1948 exceptionally favourable to take-all in this experiment and in neighbouring fields and in other parts of the country.

Weed infestation was first severe in 1947, and remained so in 1948 and 1949, indicating a cumulative effect which had become severe by the fifth year of continuous wheat cultivation.

Spraying with sulphuric acid. Only large effects were significant because, in order to avoid secondary infection after spraying, the treatment was applied to blocks, not to individual plots. Nevertheless, spraying caused strikingly significant effects, many of them greater than those resulting from any other treatment.

The benefit of spraying in 1946 appears to have been mainly due to its effect in decreasing eyespot incidence and so reducing lodging, thus increasing yield of grain and reducing the proportion of tail corn, besides reducing time and cost of harvesting. In 1947 the main benefit of spraying lay in its spectacular control of weeds, to which may perhaps be ascribed the slight increase in grain yield. The much bigger increase in grain in 1948 was probably due to the cumulative effect of spraying reducing eyespot earlier in the season than in other years, and to the control of lodging and of weeds.

Spraying in autumn inhibits sporing on bits of stubble lying on the surface of the soil, and so reduces early infection. It is less effective than, but adds to the effect of, spring spraying. While early spring spraying of suitable crops is likely to be repaying, it is uncertain whether the extra effect of autumn spraying is sufficient to justify the added expense.

Spraying in spring kills the outer infected leaves and, if applied before the fungus has penetrated deeply, leaves a healthy central part. The spray damages the crop, but improved health enables it to compensate for this and give higher yields when adequate nutrients are available. The treatment is therefore likely to be most useful on the better fertilized land, and should generally be followed by a spring dressing of nitrogenous fertilizer. Spraying with H_2SO_4 has been used commercially for control of weeds, but is then generally applied too late to control eyespot; but earlier application controls both weeds and eyespot.

Effect of sulphate of ammonia. The opposing effects of nitrogenous fertilizers on eyespot, one decreasing disease by enabling plants to produce more tillers, the other increasing disease by maintaining damper conditions more favourable to the fungus, is shown in this experiment by the decrease in eyespot incidence in May and its increase in July with added sulphate of ammonia. The balance between these opposing effects is likely to be influenced by date of application of fertilizer, a problem now under investigation.

Seed rate. Pot experiments had indicated that optimum seed rates may be lower for plants infected by eyespot or by take-all than for plants free from these diseases. In the present field experiment the most economic rates used in the three years were $1\frac{1}{2}$–2 bushels/acre, but further experiments are needed to determine the optimum rates for healthier as well as for diseased crops on this land.

General

This experiment has shown that high yields of wheat can be maintained in spite of the troubles associated with close cereal cropping. Spraying with H_2SO_4 controlled eyespot, lodging and weeds, and the addition of nitrogenous fertilizer and thin seeding both contributed to the control of take-all. Much more extensive experiments are needed under different conditions of soil, variety and climate, both when eyespot, lodging, take-all and weeds are and when they are not prevalent, to determine the conditions under which each treatment can most economically be applied.

Grateful acknowledgement is made to the many workers who helped in sampling and recording; to the farm staff; and to Mr G. M. Jolly and Mr J. H. A. Dunwoody and other members of the Statistical department for help in interpreting the data.

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EXPLANATION OF PLATE 20

Effect of sulphate of ammonia and of seed rate on lodging

(Photographs by V. Stansfield)

Fig. 1. 0 cwt./acre sulphate of ammonia (2½ bushels seed/acre).
Fig. 2. 2 cwt./acre sulphate of ammonia (2½ bushels seed/acre).
Fig. 3. 4 cwt./acre sulphate of ammonia (2½ bushels seed/acre).
Fig. 4. 1½ bushels seed/acre (4 cwt./acre sulphate of ammonia).
Fig. 5. 2½ bushels seed/acre (4 cwt./acre sulphate of ammonia).
Fig. 6. 3½ bushels seed/acre (4 cwt./acre sulphate of ammonia).

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Fig. 1

Fig. 4

Fig. 2

Fig. 5

Fig. 3

Fig. 6

THE SPREAD OF LETTUCE MOSAIC IN THE FIELD

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(With 4 Text-figures)

The epidemiology of lettuce mosaic was studied on two farms in Buckinghamshire during 1947–50. Two patterns of spread were obvious on Farm A during 1947–8: (1) spread within a crop from sources initiated by seed-borne virus; and (2) spread into a crop from nearby infected old plants. The first type caused serious losses only when aphids were unusually numerous and the incidence of seed-borne infection was high; serious losses more often resulted from the second type. Winter crops usually suffered severely, and they initiated a cycle of infection in the spring which continued from crop to crop throughout the year when aphids were sufficiently numerous. Spread from crop to crop was much reduced during 1949–50 by planting or drilling lettuce in large blocks, well separated from each other, and by omitting a winter crop. Killing the infesting aphids with an insecticide did not prevent the introduction of virus from nearby diseased crops. The insecticidal treatment of old diseased and aphid-infested crops (but before alatae are produced) is recommended.

More diseased plants often occurred near hedges, woods, trees or buildings than in the open field, presumably because such barriers halted flying aphids.

Three species of aphids were common on lettuce: *Nasonovia ribis-nigri* (Mosley), which infests the heart leaves; and *Macrosiphum euphorbiae* (Thomas) and *Myzus persicae* (Sulzer), which infest the outer leaves. *M. euphorbiae* and *M. persicae* are vectors of lettuce mosaic, but the former is less effective than *M. persicae* which, although rarely as numerous as the other two species, is the most important vector. All three species overwintered on lettuce, and migrated from the winter crops during April–May.

One obvious need to control lettuce mosaic is the production of virus-free seed. Without this, however, the disease can be ameliorated by adopting these measures whenever practicable: lettuce crops should be planted or drilled in large blocks, well separated from each other; diseased crops and crop-remains should be destroyed as soon as possible; winter crops should be treated with an insecticide in late November, and insecticides should be used to prevent the production of large aphid populations, especially on old crops.

Large acreages of lettuce are grown in the Thames valley in Buckinghamshire, and losses of about one-quarter of the plants from lettuce mosaic virus are regarded as normal; occasionally whole crops are rendered useless. The epidemiological methods used in studying potato virus diseases (Doncaster & Gregory, 1948) were applied to lettuce mosaic on two farms in this area in an attempt to arrive at control measures

by varying cultural practices. It was found that the rapid spread of the virus was usually the result of successive adjacent or nearby plantings throughout the year.

Lettuce mosaic was first recorded as a virus disease in England by Ogilvie & Mulligan (1931), and was later studied by Ainsworth & Ogilvie (1939). In the west of England, mosaic was most important in winter lettuce, and the disease was not usually common in summer crops. In the Thames valley during recent years, spring and summer crops often suffered badly, in addition to winter lettuce. Ainsworth & Ogilvie found that about 6% of the seeds from mosaic-diseased plants produced infected seedlings, and that groundsel (*Senecio vulgaris* L.) and prickly sow-thistle (*Sonchus asper* Hoffm.) were susceptible to the virus and might therefore act as sources. Sweet pea (*Lathyrus odoratus* L.) and garden pea (*Pisum sativum* L.) were also susceptible.

Lettuce mosaic is one of the few viruses which is regularly seed-transmitted. Infected plants set fewer seeds than healthy ones, but up to 15% (Kramer, Orlando & Silberschmidt, 1945) of the viable seed set by infected plants may give rise to diseased plants. Except for the variety Cheshunt Early Giant, which always gave healthy plants, germination tests at Rothamsted (Kassanis, 1947) with samples of commercial seed have usually given 1–4% infected seedlings. The plants produced from infected seed are small and valueless, but the direct loss of crop thus caused is relatively small. Their importance is in providing sources of the virus scattered throughout the crop, from which spread can occur to nearby healthy plants.

Ainsworth & Ogilvie tested a number of aphids for transmission of lettuce mosaic virus in the glasshouse and found that the only efficient vector was *Myzus persicae* (Sulzer). They obtained one infection in twenty-three plants with *Macrosiphum gei* Koch (presumably = *M. euphorbiae* (Thomas)). No transmission was obtained with *Dactynotus sonchi* (L.) (= *Macrosiphum sonchi* (L.)), *Aulacorthum solani* (Kalt.) (= *Myzus pseudosolani* Theob.) or with the common lettuce aphid *Nasonovia ribis-nigri* (Mosley) (= *Macrosiphum hieracii* (Kalt.) = *Myzus lactucae* (Schrank)), although Ainsworth & Ogilvie quote K. M. Smith as transmitting the virus to about a quarter of his test plants with *Myzus lactucae*, which is presumably *N. ribis-nigri*. Kramer *et al.* found that the virus was transmitted by *M. persicae*, *M. sonchi* and *M. solanifolii* (Ash.) (= *M. euphorbiae* (Thomas)) which had the relative efficiencies of 100:96:47. Kassanis (1947) tested several species, including *N. ribis-nigri* and *A. solani*, but only *M. persicae* caused infections. We have confirmed that *M. euphorbiae* is a vector of the virus, but found it to be inefficient compared with *M. persicae*.

Ainsworth & Ogilvie stated that *M. euphorbiae* was the commonest aphid on lettuce in the west of England, and that *D. sonchi* and *N. ribis-nigri* occurred occasionally. They never found *M. persicae* and concluded that it was not the principal vector of lettuce mosaic virus in Britain. *M. persicae* is found on lettuce in the Thames valley and is the principal vector of the mosaic virus there.

Descriptions of the farms surveyed

Both of the farms on which the lettuce crops were studied specialized in market-garden crops and were close to residential areas on the old flood plain of the River Thames in Buckinghamshire. They were about $7\frac{1}{2}$ miles apart. There were many sources of aphids on and near both farms. Overhead irrigation was used during dry weather.

Farm A (Fig. 2) had a total area of 425 acres, with few trees except around the buildings and the margins of the farm. The lettuce variety Imperial was grown as a winter crop, Improved Green Trocadero and Lobjoit's Dark Green Cos as summer crops, first planted from frames and later drilled. The rows of lettuce were 12 in. apart; the plants were spaced 10–12 in. apart in the rows.

Farm B (Fig. 3) had a total area of 260 acres, with numerous trees and some orchards; a tree-lined river ran through it. Cultural practices were similar to those on Farm A. The varieties Imperial and Winter Crop were grown during the winter, Borough Wonder, May King, Market Favourite, Northern Queen, Improved Green Trocadero and Lobjoit's Dark Green Cos during the summer.

Methods

In the initial work plants were recorded as healthy or infected, but it became obvious that more information could be obtained by grading the diseased plants according to the severity of symptoms, either mild mosaic or severe mosaic with stunting. Plants in the second grade either grew from infected seed or were infected in the young seedling stage. Symptoms were most easily seen in warm, dry weather.

The data were obtained in the following ways:

(*a*) *Spread of virus from crop to crop.* This was usually assessed by counting the diseased plants in groups of 50 along rows from one side of a crop to the other (usually 500–1000 plants). Where crop-to-crop spread had occurred infected plants predominated towards the outside of the crop, usually on the side nearest to the old diseased crop.

(*b*) *Spread of virus within the crop.* Two methods of assessing local spread were used: diseased plants were marked as soon as they were identified and records were made of neighbouring plants which became infected later; or old infected plants were transplanted into young crops, and the spread of virus around them was recorded. Occasionally the health of every plant along the row was recorded, and van der Plank's (1946) method for estimating the number of random groups of adjacent diseased plants was used to indicate whether or not there was spread from plant to plant within the crop.

(*c*) *Counts of aphids on plants.* Preliminary observations showed that the aphid infestation of individual plants varied considerably, but counts on twenty plants, taken at random across the field, provided a reasonable estimate of the mean

infestation by different species of aphids. Movement by colonizing alatae must be considerable, for infestations were often fairly uniform in any one field.

(*d*) *Aphid trapping.* Sticky aphid traps (Broadbent, Doncaster, Hull & Watson, 1948), painted bright yellow, were operated in one or two lettuce crops on each farm, with the base of the trap 9 in. from the soil. The trap covers were changed at weekly intervals during the summer, fortnightly during the winter. Catches of lettuce-infesting aphids are given in the Appendix.

Aphids infesting lettuce

Three species of aphids occurred regularly on lettuce at both farms. The most numerous was *N. ribis-nigri*, which infested the inner leaves of lettuce; next in abundance was *M. euphorbiae*, usually on the outer leaves, as was the third common species, *M. persicae*. Other species occasionally found were *Byrsocrypta bursarius* (L.) on both farms, *Acyrthosiphon* (*Lactucobium*) *scariolae* Nevsky on flower heads at Farm B, *A. solani*, *Myzus ascalonicus* Doncaster and *Hyperomyzus lactucae* (L.) at Farm A.

The numbers of aphids present on the lettuce crops, and the relative proportions of different species, fluctuated considerably during the period of survey. In November 1947, *M. euphorbiae* was numerous (10–50 per plant), but *M. persicae* and *N. ribis-nigri* were few (< 10 per plant), whereas the following April, *N. ribis-nigri* was very numerous (> 50 per plant), and the other two species were numerous, especially on the overwintered lettuce on plot 24. There was little movement by alatae before the end of April 1948, but during May large numbers from the overwintered lettuce colonized the young crops (e.g. plot 26 was colonized from plot 24). *N. ribis-nigri*, in particular, developed rapidly on the young crops, and there was another migration of alatae during the second and third week of June. During July and August aphids on lettuce became fewer, especially on older crops, but small populations developed on each young crop as it appeared.

During October and November, *M. persicae* re-infested the lettuce, presumably from Brassica and other crops on the farm. Hymenopterous parasites, syrphids and coccinellids were fairly common during the late summer and autumn, and continually checked the aphid populations. Thus a large population of *M. persicae* developed on plot 15 during October, but these aphids were destroyed by syrphid larvae during the first half of November. With the onset of winter, *M. euphorbiae* again predominated. On plot 21 all three species were found during November, January and early March; alatae and nymphs of alatae were seen on each occasion, but no alatae were trapped until April. In mid-April *M. euphorbiae* and *N. ribis-nigri* each exceeded 50 per plant on plot 21; many *M. persicae* and a few *A. solani* were also present. This crop, badly damaged by aphids, and with most plants virus-infected, was unsaleable and was ploughed in on 2 May 1949. In addition to those on lettuce, many *M. persicae* overwintered on this farm on spinach, spring greens and Cape broccoli, and

in a mangold clamp; *M. euphorbiae* also overwintered on spinach. By the end of May the aphids on the young lettuce crops often exceeded 100 per plant and alatae were being produced. Many *M. persicae* and *N. ribis-nigri* were trapped during May and June, especially in the drilled lettuce on plots 30–33. The lettuce crops were dusted with an insecticide in June, and by early July aphids were few. Syrphid larvae were numerous and soon destroyed the remaining aphids; they quickly destroyed the aphids which colonized the later drillings on the farm.

The survey was suspended during the late summer and autumn of 1949, but during the winter and 1950 it was continued on Farm A and observations were made also on Farm B. No winter lettuce was grown on Farm A, and fewer aphids overwintered on other crops. Plot 24 carried spinach which, as in the previous year, was the only overwintering source of aphids on the eastern part of the farm, but there were only three *M. persicae* and eight *M. euphorbiae* on fifty leaves of spinach on 17 April 1950, compared with 500 *M. persicae* and 80 *M. euphorbiae* on 9 March 1949. Many of the predators which developed during 1949 overwintered, and many coccinellids were seen during the spring of 1950 in the lettuce and various Brassica crops. Moderate infestations of *M. euphorbiae* and *N. ribis-nigri* developed during May in Cos lettuce on plot 14 and on drilled Cabbage lettuce on plots 30–33. These last crops were dusted with an insecticide on 6 June. Aphids developed rapidly on all the young crops in mid-June and were dusted with an insecticide about 20 June. By early July there were few aphids on these lettuces, but small populations of the three common species were developing on younger crops. Insecticide and predators almost cleared the farm of aphids; none could be found on any lettuce crop during the last half of July, and none was trapped from 19 July to 6 September. During the second half of August a few aphids colonized the younger lettuce crops, and by early October infestations were increasing again.

Observations on Farm B started on 1 October 1949, when small seedlings of winter lettuce (plot 5), adjacent to infested old Cos lettuce (plot 4), were found to be infested with an average of five *M. persicae*, eleven *N. ribis-nigri* and three *M. euphorbiae* per seedling. A trap erected between the two plots caught many *M. persicae* during the next fortnight (Appendix), but after 24 October no aphids were trapped. The lettuce from plot 5 was transplanted to plot 17, but other winter lettuce on plots 1 and 2 remained unthinned throughout the winter. These plots were very weedy, and average counts of twelve *N. ribis-nigri* and five *M. euphorbiae* per plant were made in mid-January; the weeds may have provided protection against frost. Although the transplanted lettuce had been dipped in nicotine solution, a few aphids must have survived the treatment, for a large aphid population developed on plot 17 in the spring. In mid-March the lettuce on plot 2 were ploughed in and plot 1 was cultivated and thinned. By the beginning of May the plants were heavily infested, especially with *M. euphorbiae* and *N. ribis-nigri*, despite the presence of many coccinellids. The crop was disk-harrowed on 18 May, but parts of plants infested with aphids still showed on the surface on 30 May.

During May many winged aphids colonized the younger lettuce crops. The seedlings on plot 9 were sprayed with HETP (a commercial preparation of tetraethyl pyrophosphate) on 20 May, but aphids from plot 17 continued to colonize the plants until the remains of the older crop were ploughed in at the end of May. Drilled lettuce on plot 3 were also sprayed with HETP, three times between 20 May and 27 June, but small numbers of alatae colonized the plot throughout June. Coccinellids were numerous, and soon they destroyed the aphids not killed by insecticidal treatment, for none was found in mid-July anywhere on the farm, and none was trapped during July and August.

A number of points of general interest arise from this survey. Lettuce crops provide a satisfactory overwintering habitat for the three common lettuce-infesting aphids; they are probably more important as sources of *N. ribis-nigri* in the spring, except after severe winters, than blackcurrent and other *Ribes* spp. Lettuce and spinach crops provide major overwintering sites for *M. euphorbiae*, and are as important, in such market-garden areas as the Thames valley, as Brassica crops for overwintering *M. persicae*.

During the three years of this survey, aphid populations on lettuce crops were maximal during May, and minimal during July and August. Predators and parasites were partly responsible for the paucity of aphids during the summer, but the heat near the soil surface on sunny days may also have been detrimental, for Fenjves (1945) found that *M. persicae* did not flourish at temperatures of 82° F. and above.

Parasites and predators became less active during the autumn, and aphid populations increased again. Syrphid larvae, when present, remained active into the winter. Aphids continued to reproduce when temperatures were above 40° F. (Fenjves, 1945), and alatae often developed, but as there is little or no flight at temperatures below 55° F. (Broadbent, 1949) flight would usually be impossible from November to March.

M. euphorbiae were not trapped in large numbers, though they were common on the lettuces; apterae of this species are very mobile, and often walked on to the sticky traps.

Seed infection and the spread of virus within the crop

The cabbage lettuce seed used on both farms gave 1% or less virus-infested seedlings (usually about 0·5%), but the Cos lettuce usually gave about 3%. The spread of virus from the widely scattered plants arising from these infected seeds was largely confined to nearby plants, so that small patches of infected plants developed. Fig. 1 shows two typical patterns of virus infection around plants which developed from virus-infected seeds. The tendency for plants adjacent or near to the infected plant to become infected is shown. Although the numbers of infected plants in the two areas depicted are somewhat similar at distances of one to four plant spaces from the infectors, totalling 14, 10, 12 and 10, the total number of plants increases with the distance from the source, and the percentage infected falls from 88 to 33, 26 and 17.

During 1948 some information on local virus spread was obtained by recording the disease around older virus-infected plants transplanted into plots 6–8 weeks before harvest. These conditions were artificial, but the experiments showed that most new infections were close to the diseased source.

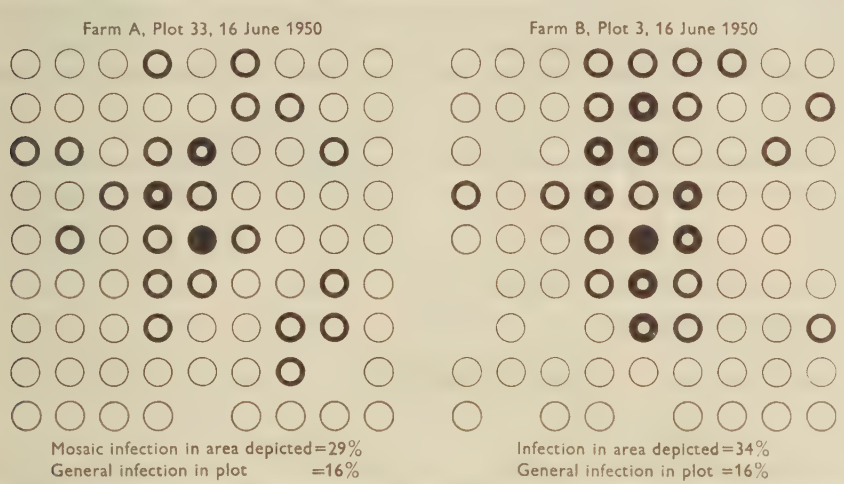
Farm A, Plot 33, 16 June 1950
Farm B, Plot 3, 16 June 1950
Mosaic infection in area depicted=29%
General infection in plot =16%
Infection in area depicted=34%
General infection in plot =16%

Fig. 1. The distribution of infected plants around plants from seed infected with lettuce mosaic virus. ●, Primary infector. ◉, Secondary, early infection. ○, Secondary, late infection. ○, Healthy.

TABLE 1. *Local gradients of infection in lettuce around old virus-infected plants*, 1948

Plot	Ten 'infectors' transplanted	Date recorded	Percentage plants infected in plant position from 'infector'* 1	2	3	4	5	6
26	14 Apr.	26 May	20	16	16	7	7	3
17	26 May	7 July	32	7	7	7	7	2
32	7 July	25 Aug.	20	67	6	0	0	2
11	25 Aug.	6 Oct.	5	0	0	0	0	2
		Mean	19	23	7	4	4	2

* Based on counts of twenty plants in each position.

When infection was confined to this local spread, the incidence of lettuce mosaic at harvest depended on the numbers and mobility of the aphids infesting or visiting the crops. The crops listed in Table 2 were grown from seed about 0·5% virus-infected. The numerous aphids in May and June 1949 caused an increase in infection on plot 33 (Farm A) to 14%, but in the Cos lettuce (plot 8), which was about 3% seed-infected, mosaic incidence rose to 85%. Thus the initial percentage infection in both crops was increased by a factor of 28. This might have been coincidental, but it demonstrated clearly that a crop with 3% initially infected plants is likely to fail completely, whereas one with 0·3% will be satisfactory under similar

conditions of aphid activity, and it emphasizes the great importance of reducing seed infection to a minimum.

The biggest spread of virus from seed-infected sources was in plot 19 (Farm A) during June 1950. No trap was operated in this plot, but many *M. persicae* were noted on the plants, presumably coming from adjacent crops of spinach, turnip and red beet. There was no evidence of the introduction of virus from outside, there being rather less mosaic towards the edges of the plot. The incidence of virus rose from 1 to 34%. When the aphid infestation of the plants was noticed, an application of an insecticidal dust, containing DDT and nicotine sulphate, was made on all the lettuce crops on the farm. This killed the aphids or forced them to move elsewhere, and the great increase in mosaic incidence might have been the result of activity by alatae, irritated by the insecticide. The examples given in Table 2 show that much more virus was transmitted by fewer aphids during the spring of 1950 than in 1949, when insecticides were not used.

TABLE 2. *Alate aphids trapped, and the incidence of lettuce mosaic in crops of Trocadero lettuce at harvest (crops in which there was no evidence of spread from outside)*

Date	Plot	Aphids trapped during growth of crop			Percentage of plants infected with lettuce mosaic virus at harvest
		M.p.	*N.r.-n.*	*M.e.*	
Mar.–May 1948	Farm A 3	14	15	2	4
Mar.–May 1949	2	15	40	11	2
Mar.–May 1949	3	23	53	11	3
Apr.–June 1949	33	107	605	8	14
Mar.–May 1950	2–4	4	0	0	3
Mar.–June 1950	30–33	19	36	2	16
July–Aug. 1950	6	2	4	0	3
July–Sept. 1950	31	0	0	0	4
Sept. 1950	27	2	0	0	1
	Farm B				
July–Aug. 1950	12	3	5	0	0·4
July–Aug. 1950	18				0·6
July–Sept. 1950	7	1	2	1	2

THE SPREAD OF VIRUS FROM CROP TO CROP

Most of the lettuce crops became infested with aphids when young, but as the crops matured the populations became alate and migrated to younger crops. During their colonization of the crops the aphids spread virus from the plants which arose from infected seeds, so that when the aphids moved away a proportion of them carried virus to the younger crop. Those plants infected soon provided 'internal' sources of virus, additional to those arising from seed infection.

This crop-to-crop spread was well illustrated on Farm A during 1948 (Figs. 2 and 4). In November 1947 plots 2 and 7 carried lettuce crops, most plants of which

were virus-infected. Winter lettuce was drilled on plot 1 in September and transplanted to plots 13, 21 and 24, where it overwintered. Infection was apparently acquired in the seed-bed from plot 2, for in April plot 21 was so badly infected (60% dead, 90% of the surviving plants showing mosaic) that the crop was ploughed in. Of the plants in plot 13, 26% had mosaic symptoms, of those in plot 24, 52% were infected and there was no evidence that virus spread had occurred after transplanting (van der Plank test).

Fig. 2. Plan of Farm A. Fields which carried lettuce are numbered.

During March plots 8 and 16 were planted with Cos lettuce from frames; 3–5% of the plants were infected at the beginning of May, but the numerous aphids leaving winter lettuce (e.g. plot 13) probably carried more virus to the Cos, for by mid-June 41% of the plants in plot 8 and 24% of those in plot 16 had mosaic symptoms.

Drilled Trocadero lettuce on plot 17 germinated at the end of April, and by the end of May large populations of aphids had developed on them. In mid-June 1% of the plants was infected, but by 7 July, although only 2% of the plants in the centre of the plot were infected, 93% were infected on the north side against the Cos lettuce in plot 8. On the same date in later-sown Cos lettuce in plot 9, 3% of the

plants in the centre of the plot were infected, but 40% on the western side, adjacent to plot 8. Plot 20 was drilled during July; on 25 August 15% of the central plants were infected, compared with about 50% of the plants near the edges.

The practice of planting or drilling lettuce on any empty land, without reference to the proximity of other lettuce crops, was obviously a principal cause of severe loss from lettuce mosaic virus. An attempt to overcome this difficulty was made during the spring of 1949, when all the Trocadero lettuces from frames were planted in one block on plots 2–3; the Cos lettuce was planted on plot 8. The only overwintering

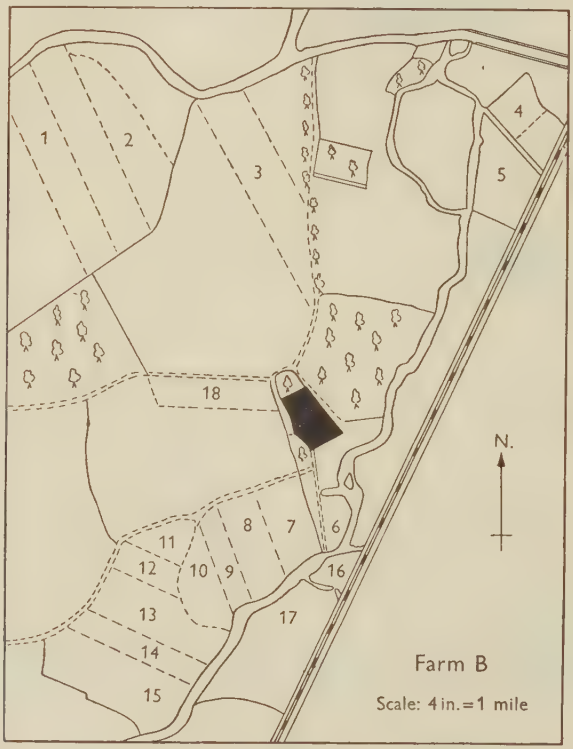

Fig. 3. Plan of Farm B. Fields which carried lettuce are numbered.

lettuce was on plot 21; a large aphid population developed on these plants during April, but the crop was ploughed in at the end of April, before many aphids had migrated from it. On 2 May there was an average of four aphids per plant (mainly *N. ribis-nigri*) on the lettuce on plots 2–3, and although multiplication was rapid and large populations developed during May, the spread of virus was from infected seedlings only; thus 2% of the plants in the earlier plantings were infected at harvest (16 May) and 5% in the later plantings (31 May). The more slowly maturing Cos

lettuce on plot 8, however, starting with 3% plants infected from seed, reached 85% of plants infected by 30 June, and drilled Cos lettuce on the eastern part of plot 8, and plot 17 also became badly infected. Evidence of severe losses from lettuce mosaic virus in the Thames valley in 1949, when spring crops were planted near winter lettuces, is given by Last (1950).

The spring-drilled Trocadero lettuce was planted in one block on plots 30–33, as far away as possible from growing crops. The early drillings became infested with aphids in April and the populations developed rapidly during May and June; however, there was no nearby source of virus, and the only spread was from infected seedlings. Later drillings were made on plots 4 and 12, and the plants emerged before the old plants on plots 3 and 8 had been destroyed. Virus was carried to the young crops, plot 4 had 14% and plot 12 had 13% plants infected on 30 June.

Winter lettuce was not grown on Farm A during 1949–50, so there was no source of lettuce mosaic virus, except for seed infection (there was no evidence that infected weeds occurred or played any part). The spread of virus was compared with that on Farm B (Fig. 3), where three winter lettuce crops were grown.

On Farm A the planted lettuce was all on plots 2–4, the drilled on plots 30–33, similar to the 1949 plan, but the Cos lettuce was planted farther away, on plot 14 (Fig. 4). On 30 May 6·5% of the Cos plants were infected (the Cos seed this year gave about 1·5% virus-infected seedlings). Cos lettuce was also drilled on plot 14, next to the planted Cos; on 30 May the younger Cos had 2% infected plants, but by 16 June, when it was mature there were 19%, and infection was much worse near the edges of the plot; for instance, 90% of the plants in the two rows near the western headland were infected. There was no evidence of introduction of virus from outside in either the planted or drilled blocks of Trocadero lettuce.

No lettuce was drilled on the northern part of the farm until the planted block on plots 2–4 had been cleared. Plots 12, 19 and 22–23 germinated in early June; infection in plots 19 and 22–23 was limited to spread from infected seedlings, but plot 12 obviously received infection from outside: on 11 July 19% of the plants in the third row from the north, 9% of those in the middle and 4% of those in the third row from the south were infected. Across the middle of the plot the incidence of mosaic rose from east to west, counts per 100 plants being 8, 2, 5, 8, 18, 35, suggesting that the virus was coming from the Cos lettuce on plot 14.

Conditions on Farm B were very different (Fig. 4). Here lettuce overwintered, and no attempt was made to isolate the successive crops. Part of the winter lettuce on plot 17 had failed and had been replaced by plants from plot 2. In the spring 7% of these showed mosaic, compared with 40% of the plants which had been transplanted from plot 5, adjacent to the diseased Cos lettuce on plot 4. The badly diseased portion of the field was also more heavily infested with aphids than the other. The winter lettuce on plot 1 also had about 7% plants infected.

Plot 7 was planted with lettuce from frames during the last week of March, plot 6 with Cos lettuce. On 2 May four plants of 1300 cabbage lettuce examined had mosaic

symptoms, but on 30 May 1% of the plants showed severe, and 10% mild symptoms. Of the Cos plants 6·5% had severe and 15% mild symptoms. It is probable that these late infections were caused by infective aphids from plot 17. In plot 9, however, on 30 May 8% of the plants had severe, and 53% mild symptoms. Mosaic incidence was worse here than on plot 7, possibly because the plants were younger at the time of infection and the plot was opposite the worst part of plot 17. Three

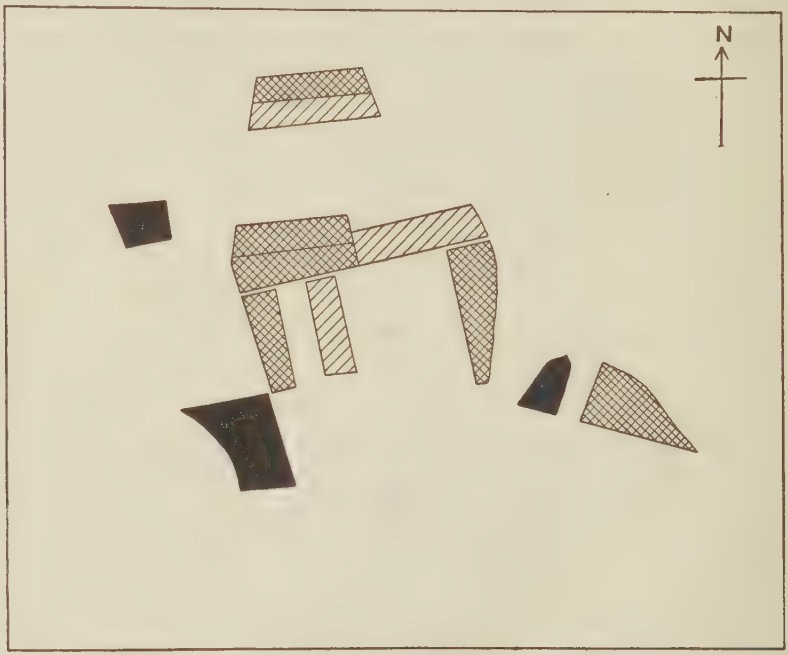

Fig. 4(*a*). Farm A, spring, 1948. ■, winter lettuce; ◈, lettuce planted from frames; ▨, drilled lettuce.

strips of lettuce were drilled also on plot 3, at 10-day intervals from 29 March. On 16 June 6·5% of the first drilling showed severe, 10% mild, mosaic; of the second drilling 10% had severe, 29% mild, mosaic. On 27 June 20% of the second drilling had severe, 25% mild, mosaic; of the third drilling 2% had severe, 23% mild, mosaic. Thus it would appear that infection had occurred over a period, the first wave when the first and second drillings were small and susceptible, and the third not through the ground. The third drilling may have been infected from the older plants in the plot, but not by aphids bred thereon, for large aphid populations were prevented from developing by insecticidal treatment. No aphids were found, nor was there any increase in disease incidence, on the third drilling, when examined on 11 July. There were no obvious 'gradients' of infection from the edges of the plot, but some infection was probably carried to these long narrow strips from the remains of the diseased winter lettuce on plot 1.

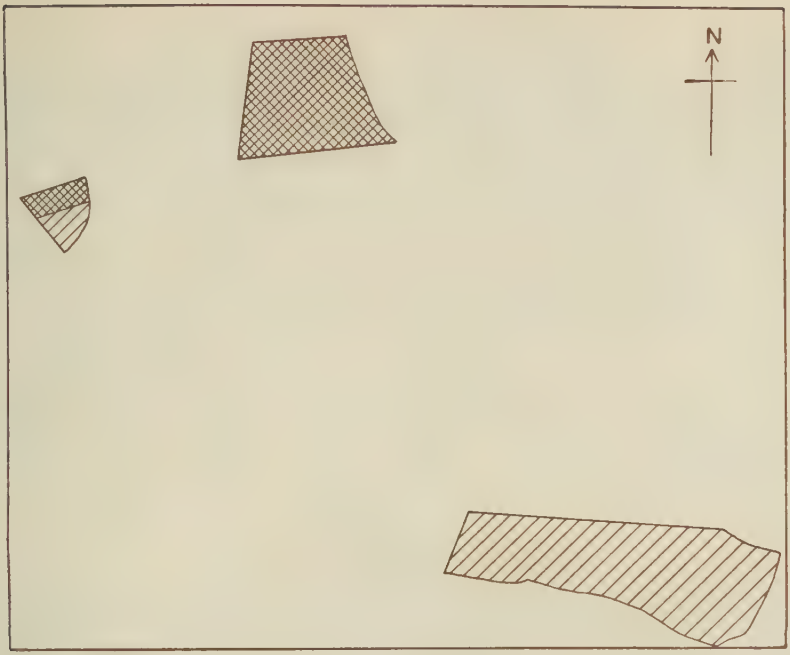

Fig. 4(*b*). Farm A, spring, 1950. ■, winter lettuce; ▨, lettuce planted from frames; ▨, drilled lettuce.

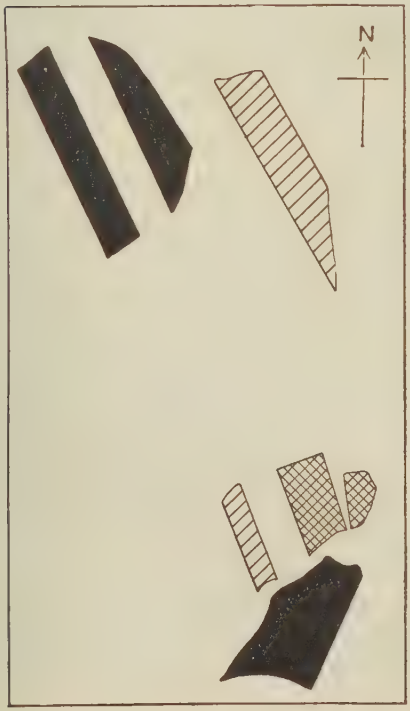

Fig. 4(*c*). Farm B, spring, 1950. ■, winter lettuce; ▨, lettuce planted from frames; ▨, drilled lettuce.

Fig. 4 shows the distribution of lettuce crops when much crop-to-crop spread of virus occurred (Farm A, 1948; Farm B, 1950), compared with a distribution (Farm A, 1950) when spread was mainly local from infected seedlings. To have been completely successful the Cos lettuce planted and drilled on the western part of Farm A in 1950 ought to have been separated. Table 3 gives the incidence of lettuce mosaic in crops of similar age on the two farms in 1950.

TABLE 3. *A comparison of lettuce mosaic incidence in drilled crops of comparable age when isolated (Farm A) and when near old crops (Farm B)*

	Percentage plants with severe or mild mosaic symptoms					
	Farm A			Farm B		
Date	Plot	Severe	Mild	Plot	Severe	Mild
30 May 1950	30	0·5	3	9	8	53
16 June 1950	33	4	12	3*	10	29

* Second drilling.

DISCUSSION

There are two causes of severe loss from lettuce mosaic virus, the first is seed infection, the second is the practice of growing young crops near or adjacent to older crops, which are often aphid infested and diseased. There was evidence that young plants were more susceptible than old; when late infection occurred, the plants were often marketable, whereas early infection always resulted in unsaleable plants. As in the west of England (Ainsworth & Ogilvie, 1939), winter lettuce suffered most, for these crops were open to infection during autumn, perhaps through the winter, and in spring. With rapid growth in late spring and summer, the time during which crops might become infected was shortened.

If nearby infected crops, especially of winter lettuce, are important sources of virus, what is the minimum distance which will provide adequate protection? This question is difficult to answer, for local conditions, such as tree barriers and intervening crops, will affect aphid flight. Substantial reduction of mosaic was obtained on Farm A during the spring and early summer of 1949 and 1950, for it was not prevalent although aphids were abundant, and other crops in the district suffered severely. Farms in the Thames valley usually grow a considerable acreage of cruciferous crops, and if these are placed between blocks of lettuce it is probable that migrating *M. persicae* will feed on them, even if they ultimately reach the next lettuce block. As lettuce mosaic virus does not persist in its vectors, either feeding on another host, or a long flight, will result in the virus being inactivated in the aphid, and the chances of infective aphids reaching a new lettuce crop will decline with increasing amount of intervening land under crops.

A striking feature of the crop-to-crop spread was the concentration of infected plants in the outer rows of the crop, almost all the plants in these rows sometimes being infected. This is a common phenomenon with aphid-borne viruses, even

though the vectors are mobile and gradually disperse over the field. Colonizing aphids often infest the edge plants in a crop, especially those species which tend to remain on the first suitable host they find, e.g. *Aphis fabae* Scop. (Johnson, 1950). An aphid carrying a non-persistent virus is likely to infect the first susceptible plant upon which it feeds, and its chances of later infecting other plants on which it may feed rapidly diminish. The greater the area of crop, the smaller the proportion of the crop formed by the infected edge rows, and less economic loss is likely in a large block of lettuce than in a small, both being subject to the same number of infections.

It was often noticed that the percentage of infected plants increased near hedges or trees, even though these might be at the side of a plot farthest from the source of infection. Trees or hedges act as barriers to aphid flight, and some of those aphids which did not fly over would settle on the lettuce nearby; this would be more likely on windy days when aphids were being transported involuntarily over the crop.

N. ribis-nigri is not a vector of lettuce mosaic virus, but as it is the most numerous aphid and infests the inner leaves of lettuces, it is the principal pest. *M. persicae* is the most important virus vector (Ainsworth & Ogilvie, 1939; Kassanis, 1947) and is sufficiently numerous to account for most of the virus spread. It is this aphid which, during its autumn migration, probably infects the young winter lettuce crop. *M. euphorbiae* is a relatively inefficient vector, but is often numerous on lettuce during the winter, and its apterae are very mobile; it may play a part, therefore, in spreading virus throughout the winter crop. Alate *M. persicae* are probably the main carriers of virus from the winter to the spring crop, and from crop to crop during the spring, summer and autumn.

Control measures

1. The first obvious remedy is the production of virus-free seed. It would take some years to eliminate the reservoir of virus in the lettuce crops, but new sources would not be planted at frequent intervals as at present. With adequate isolation, and careful inspection, virus-free seed crops ought to be produced.

Until virus-free seed is generally obtainable, growers can ensure that they use only the best by growing a few hundred seedlings before using a stock of seed in the field. More than 0·5% of infected seed may lead to a serious loss of crop.

2. Frames in which seedlings are to overwinter, or seed-beds from which winter lettuce are to be transplanted, should not be close to other lettuce crops. Crops of lettuces of similar ages should not be scattered about the farm; they should be in blocks, separated from each other by as great distances, and as many other crops, as possible. New drillings or plantings should never adjoin much older crops. Open sites are preferable to enclosed ones; seed-beds, in particular, should be placed away from hedges, woods or buildings.

3. The remains of all lettuce crops should be ploughed in as soon as possible. It is a bad practice to leave diseased lettuce when the healthy are being cut, but cutting and leaving to wilt will not destroy the aphids, but will encourage them to move.

4. When transplanting, lettuces should be dipped in a nicotine solution. Winter lettuces should be treated with an insecticide during late November, when the autumn migration is over, to prevent apterous aphids spreading virus during the winter, and the development of a large number of alatae during the spring. Any crop which becomes badly infested with aphids should be treated with an insecticide, although treatment of a young crop will probably not prevent virus infection. The aphids should be destroyed on the old, infected crops before they produce large numbers of viruliferous alatae.

We are indebted to Dr P. H. Gregory and Dr B. Kassanis who helped to initiate this work and made some of the observations during the first year. We also thank the farmers, Mr H. Rochford and Mr W. G. White, and their managers Mr A. T. Enticknapp and Mr J. W. Davies, for permission to work on their crops and for much assistance, including the regular changing of the aphid traps.

APPENDIX

Alate lettuce-infesting aphids trapped

	Date	Plot	*M. persicae*	*N. ribis-nigri*	*M. euphorbiae*
		Farm A, 1948–50			
1948	13 Feb.–	21	—	—	—
	17 Mar.		0	0	0
	24 Mar.		1	0	0
	31 Mar.		0	1	0
	14 Apr.		0	0	0
1948	14–28 Apr.	26	2	2	1
	5 May		5	4	1
	12 May		17	11	3
	19 May		15	26	0
	26 May		2	15	1
1948	5–12 May	3	8	0	1
	19 May		5	4	0
	26 May		1	11	1
	2 June		0	0	0
	9 June		3	5	1
	16 June		0	34	3
1948	26 May–	17	—	—	—
	2 June		0	1	0
	9 June		5	6	6
	16 June		4	25	3
	23 June		2	47	2
	30 June		4	4	0
	7 July		0	0	7
1948	7–21 July	32	1	0	5
	28 July		0	1	2
	4 Aug.		0	0	1
	11 Aug.		0	1	0
	11–25 Aug.		0	0	0

	Date	Plot	*M. persicae*	*N. ribis-nigri*	*M. euphorbiae*
1948	25 Aug.–	11	—	—	—
	15 Sept.		0	0	0
	22 Sept.		0	1	0
	29 Sept.		0	1	1
	6 Oct.		3	5	0
	13 Oct.		3	0	0
	13 Oct.–3 Nov.		0	0	0
	10 Nov.		1	0	0
	17 Nov.		1	0	0
1948	17 Nov.–	21	—	—	—
	1 Dec.		0	0	0
	8 Dec.		0	1	0
	15 Dec.		0	0	1
	No aphids trapped during the winter until				
1949	6 Apr.		0	0	4
	20 Apr.		9	39	7
1949	2 May–	3	—	—	—
	11 May		6	1	0
	27 May		8	13	0
	13 June		11	100	2
	23 June		3	17	0
1949	2 May–	33	—	—	—
	11 May		35	0	1
	27 May		23	21	2
	13 June		22	473	3
	23 June		27	111	2
1950	10 Mar.–	2	—	—	—
	2 May		0	0	0
	12 May		4	0	0
1950	22 Mar.–	30	—	—	—
	31 Mar.		0	0	1
	2 May		0	0	0
	12 May		1	0	0
	13 June		18	36	1
1950	17 June–	23	—	—	—
	11 July		9	14	0
1950	11 July–	3	—	—	—
	19 July		1	1	0
	6 Sept.		0	0	0
	13 Sept.		0	1	0
1950	11 July–	31	—	—	—
	6 Sept.		No lettuce-infesting aphids trapped		
1950	6 Sept.–	29	—	—	—
	13 Sept.		1	0	0
	22 Sept.		0	0	0
	27 Sept.		1	0	0
		Farm B, 1949–50			
1949	1 Oct.–	5	—	—	—
	8 Oct.		27	3	0
	15 Oct.		33	4	3
	24 Oct.		9	2	4
	12 Nov.		0	0	0
1950	12 Jan.		0	0	0

	Date	Plot	*M. persicae*	*N. ribis-nigri*	*M. euphorbiae*
1950	12 Jan.		No aphids trapped until		
	15 Apr.	1	1	0	2
	22 Apr.		2	3	3
	2 May		6	14	2
	8 May		8	2	3
	13 May		13	1	6
1950	22 Mar.–	7	—	—	—
	15 Apr.		1	0	0
	8 May		0	0	0
	13 May		10	0	0
	20 May		5	2	0
	5 June		72	29	17
1950	6 June–	3	—	—	—
	16 June		4	15	0
	26 June		7	6	0
	3 July		3	8	1
	10 July		0	0	0
	29 Aug.		0	0	0
1950	3 July–	12	—	—	—
	10 July		3	3	0
	25 July		0	1	0
	8 Aug.		0	1	0
	29 Aug.		0	0	0
1950	30 Aug.–	7	—	—	—
	20 Sept.		0	1	0
	4 Oct.		1	0	1
1950	30 Aug.–	17	—	—	—
	20 Sept.		1	1	0
	4 Oct.		0	0	1

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A VIRUS DISEASE OF RED CURRANT (*RIBES RUBRUM* L.)

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(With Plate 21)

A virus causing ringspot patterns and vein clearing of red currant leaves is reported. It is inactivated at 66–68° C., tolerates a dilution of 1:2000 but not 1:5000, loses infectivity after 7–9 days *in vitro* and has been transmitted only by sap inoculation.

The virus infects plants in a wide range of families, resulting in chlorotic and necrotic symptoms. On *Nicotiana tabacum* it causes local and systemic symptoms of the ringspot type. Its host range and physical properties differ from other viruses causing ringspot symptoms, and the name red currant ringspot virus is therefore suggested.

Introduction

During the late summer of 1948, two sets of red currant plants imported from Holland to England showed leaf symptoms suggesting that they were virus infected. This suggestion was confirmed by inoculating leaf extracts to tobacco, *Nicotiana tabacum* var. White Burley, which developed striking chlorotic patterns. As there have been very few reports of virus transmission from a woody to a herbaceous plant, the host range and properties of the virus were investigated. Tobacco plants were used as the main test plant and source of inoculum.

The first reported virus disease of red currant, *Ribes rubrum* L., was the currant mosaic described by Hildebrand in 1939 in the U.S.A. The main symptom was a chlorotic irregular veinbanding which developed from many light green spots, and also a marginal necrosis and defoliation. The plant was stunted and sometimes showed unilateral dieback or a general decline. The virus was transmitted to currant only by inarching of vigorous shoots. By 1944 this disease was widespread, and of the fifty-five varieties of currant tested by Hildebrand & Weber, thirty-five were found to be susceptible. Symptoms were usually masked by temperatures over 70° F., but occasionally plants at 70–80° F. developed chlorotic ringspot patterns on the older leaves, and zonate chlorotic spots on the immature ones (Hildebrand, 1942).

The virus was transmitted by sap inoculation to various herbaceous plants. Infected tobacco and *Nicotiana sylvestris* developed zonate necrotic lesions in 7 days. *N. glutinosa* was a symptomless carrier and *N. rustica* was killed. Young tomato plants developed concentric necrotic spots and were extremely stunted with

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string-like growth of the leaves. Infected plants set no fruit. Hildebrand failed to return the virus to red currants by any method of mechanical transmission.

Cross-immunity tests showed the virus to be related to the tomato ringspot of Imle & Samson (1937).

In Germany, Winter (1940) described a virus complex of red currants, which sometimes caused a stiff growth habit with irregular-shaped lead-grey leaves, and sometimes a drastic stunting of the long shoots with a profusion of secondary ones. These had diffusely mottled malformed leaves.

Mosaic diseases of both red and black currants, caused by undetermined viruses, were reported by Blattny (1930) in Czechoslovakia.

The naturally infected red currant plants used in these experiments had dark green leaves with vivid yellow rings, about 1 cm. diameter, and a green centre. This chlorosis caused uneven growth of the laminae.

METHODS AND MATERIALS

(*a*) *Mechanical inoculation.* Leaves from recently infected plants were macerated with a pestle and mortar, and the plant sap was lightly rubbed by a finger on leaves dusted with carborundum powder. The residual extract was then washed off.

(*b*) *Insect vectors.* Virus-free aphids were starved for approximately 2 hr. before being placed on either an infected plant or on a portion of infected leaf kept moist in a Petri dish. After feeding on both infected and healthy tissue for varying periods, they were killed by nicotine spray.

(*c*) *Grafting.* The cleft graft was used for the tobacco and the inarching method for the red currant.

(*d*) *Physical property tests.* For the thermal inactivation and dilution end-points, and longevity, the methods of Johnson & Grant (1932) were used.

All test plants, except some red currants, were grown from seed in insect-proof glasshouses. The red currant plants were of three types: (1) raised from seed; (2) cuttings rooted in the glasshouse; (3) naturally growing young plants in an orchard.

After inoculation the plants were kept in the glasshouses at varying temperatures. They were regularly fumigated with nicotine.

Properties, transmission, cross-immunity tests and host range

The thermal inactivation point, dilution end-point and longevity *in vitro* are shown in Table 2, which also gives the values for other viruses that cause ringspot symptoms in tobacco. In desiccated leaf tissue the virus was still active after 12 days but not after 15 days. It was inactivated by dilutions of 1:500 HCl and 1:500 37% formaldehyde.

Tobacco plants infected with the red currant ringspot virus were not protected against infection by tobacco ringspot. When plants systemically infected with the

first were inoculated with the second they developed typical symptoms of tobacco ringspot.

The only successful method of transmitting this virus has been by mechanical sap inoculation. It was not transmitted by *Aphis fabae* Linn., *Macrosiphum pisi* Kalt., *Myzus lactucae* Schrank. and *M. persicae* Sulz.

TABLE 1. *Comparison of the host range of the four ringspot viruses*

Host plant	Red currant ringspot	Tobacco ringspot	Tomato ringspot 1*	Tomato ringspot 2†
Amaranthaceae:				
Amaranthus tricolor	+	+	+	+
Aizoaceae:				
Tetragonia expansa	+	+	±‡	−
Chenopodiaceae:				
Spinacia oleracea	+	+	−	−
Compositae:				
Zinnia elegans	+	+	−	+
Cucurbitaceae:				
Cucumis sativus	+	+	−	+
Saxifragaceae:				
Ribes rubrum	+	−	−	+
Solanaceae:				
Datura stramonium	+	+	+	−
Lycopersicum esculentum	+	+	+	+
Nicandra physalodes	+	+	+	−
Nicotiana glauca	+	−	−	−
N. glutinosa	+	+	−	+
N. rustica	+	+	−	+
N. sylvestris	+	+	−	+
N. tabacum var. Kawala	+	−	+	+
var. White Burley	+	+	−	−
Petunia hybrida	+	+	+	−
Physalis angulata	+	+	+	−
Solanum mericatum	+	−	−	−
S. nigrum	+	+	−	−
S. nodiflorum	+	−	−	+

Host plant	Red currant ringspot	Tobacco ringspot	Tomato ringspot 1*	Tomato ringspot 2†
Chenopodiaceae:				
Beta vulgaris	−	+	−	+
Compositae:				
Calendula officinalis	−	+	−	−
Dahlia variabilis	−	−	−	−
Silybum marianum	−	−	−	−
Cruciferae:				
Brassica chinensis	−	−	−	−
B. oleracea	−	−	−	−
Cucurbitaceae:				
Cucurbita pepo	−	+	−	−
Leguminosae:				
Medicago lupulina	−	−	−	−
Melilotus officinalis	−	+	−	−
Phaseolus vulgaris	−	+	−	+
Pisum sativum	−	−	−	−
Polemoniaceae:				
Phlox drummondii	−	−	−	+
Scrophulariaceae:				
Mimulus luteus	−	−	±‡	−
Solanaceae:				
Hyoscyamus niger	−	−	+	−
Lycium barbarum	−	−	−	−
Solanum capsicastrum	−	+	+	−
S. capsicum	−	−	−	−
Tropaeolaceae:				
Tropaeolum majus	−	−	−	−

* Samson & Imle (1942). † Hildebrand (1942). ‡ Price (1940).
+, susceptible; −, non-susceptible.

The following plants have been found to be susceptible: *Amaranthus tricolor* L., *Cucumis sativus*, *Datura stramonium* L., *Lycopersicum esculentum* Mill., *Nicandra physalodes* (L.) Pers., *Nicotiana glauca* L., *N. glutinosa* L., *N. rustica* L., *N. sylvestris* Speg. & Comes., *N. tabacum* L., vars. Kawala and White Burley, *Petunia hybrida* Vilm., *Physalis angulata* L., *Ribes rubrum* L., *Solanum mericatum* L., *S. nigrum* L., *S. nodiflorum* L., *Spinacea oleracea* L., *Tetragonia expansa* Murr., *Zinnia elegans* L.

Symptom expression

Red currant (*Ribes rubrum* L.). When red currant seedlings were inoculated from tobacco, the first symptoms appeared in about 28 days as a chlorosis in the young leaves, which were then 5th or 6th above the inoculated ones. These had chlorotic rings with green centres, or irregular bands roughly following the veins, and there was also slight malformation. On the lower leaves, there were occasional chlorotic flecks along the veins or small rings. As the leaves matured, this chlorosis became vivid yellow, but no further symptoms developed in the new growth (Pl. 21, fig. 1). In the late summer, the only symptom was chlorosis of the veins of the 5th–10th leaves. Neither the leaves nor the plants were reduced in size.

TABLE 2. *Comparison of the physical properties of the ringspot viruses*

Virus	Thermal inactivation point (° C.)	Dilution end-point	Longevity	Author
Tobacco ringspot	55–60	1:1000–10,000	3–4 days	Henderson & Wingard
Tomato ringspot 1	56–58	1:250–500	21–27 hr.	Samson & Imle
Tomato ringspot 2	60	1:1000–5000	3–4 days	Hildebrand
Red currant ringspot	66–68	1:2000–5000	7–9 days	Klesser

TABLE 3. *Comparison of the symptoms on four main host plants of the ringspot viruses*

Virus	Tobacco	*N. glutinosa*	Tomato	*Petunia*
Tobacco ringspot	Local necrotic ringspots in 2–3 days. Systemic necrotic rings in 8–14 days	Local and systemic necrotic spots	Necrotic ring and line patterns	Stunt, Chlorotic mottling with necrosis
Tomato ringspot 1	Local ring and line patterns. Systemic chlorotic patterns	—	Local and systemic necrotic ring and line patterns	—
Tomato ringspot 2	Zonate necrotic lesions in 7 days	—	Zonate necrotic spots. Severe malformation	—
Red currant ringspot	Local necrotic ringspots in 6–8 days. Systemic concentric chlorotic patterns in 16–20 days	Local and systemic concentric chlorotic patterns	Local chlorotic spots. Systemic chlorotic patterns. Slight curl	Local ringspots. Systemic chlorotic patterns

No symptoms developed on either the cuttings, or the young plants which were inoculated out-of-doors.

Tobacco (*Nicotiana tabacum* var. White Burley). Local lesions appeared in 6–8 days starting as chlorotic spots and soon forming many concentric rings of yellow and green. Occasionally the rings became elongated where they crossed a leaf vein, but this was uncommon.

After 16–20 days systemic symptoms appeared on the young leaves, and also on the lower expanded leaves. The two leaves immediately above the inoculated pair

rarely showed any chlorosis, while the 3rd, 4th and 5th developed varying numbers of separate irregular patterns. These consisted of well-defined alternating lines of yellow and green tissue, and at the bases of these leaves there was usually a symmetrical patterning on either side of the midrib and extending along the first two veins (Pl. 21, fig. 5).

The markings on the 6th and 7th leaves were even more striking, being brighter yellow and more regular in outline. They followed the veins to form an oak-leaf pattern and had from two to six distinct bands, with an average of four. One or more of the lines sometimes became white and necrotic, especially near the apex of the leaf.

Later leaves were all symptomless, but the virus could be recovered from them. This was also true of the two leaves immediately above the inoculated pair.

During the winter months symptoms were never vivid; nor were there any distinct patterns, but rather a diffuse interveinal chlorosis with only occasional concentric markings. There was also less necrosis at the lower temperatures (Pl. 21, fig. 4).

The variety Kawala showed a slight local reaction and less concentric patterning with no necrosis.

N. glutinosa. Local lesions appeared in about 6–8 days as chlorotic spots with a surrounding necrotic ring. The chlorosis spread and the leaf wilted and collapsed after about 20 days. Systemic infection did not always follow, but on some plants individual concentric chlorotic patterns developed in 13–16 days. The virus could be recovered from the symptomless young leaves.

N. glauca, *N. rustica*, *N. sylvestris*, *Nicandra physalodes* and *Solanum nigrum*. No obvious local patterns were produced but only occasionally chlorotic spots. Systemic symptoms were similar to those on White Burley.

On *Nicotiana glauca* the ring patterns were more regular and never had more than two concentric bands. They sometimes formed shallow blisters.

Datura stramonium and *Solanum mericatum*. Local necrotic ringspots appeared in 14 days and were followed by isolated individual chlorotic spots on the youngest leaves.

Petunia hybrida. The local reaction was similar to that on tobacco, but on the young leaves the chlorotic rings soon became necrotic. There was slight malformation of the leaves due to the intense patterning. Later growth was extremely chlorotic (Pl. 21, fig. 2).

Physalis angulata. Necrotic local lesions appeared in 7–8 days, and systemic symptoms in 14. A severe necrotic spotting causing the young leaves to wilt and drop, leaving the bare stem.

Solanum nodiflorum. Symptoms developed only on young leaves, and consisted of chlorotic patterns with light necrosis.

Amaranthus tricolor. Symptoms were very similar to those on *Physalis angulata*.

Tomato (*Lycopersicum esculentum*). Locally there were large chlorotic blotches. The first set of leaflets were flecked along the veins with occasional rings; but on the

following leaves concentric chlorotic patterns were general, giving a mottled appearance. The young leaflets tended to curl downwards, and on mature plants they were twisted and symptomless (Pl. 21, fig. 3).

New Zealand spinach (*Tetragonia expansa*). Both local and systemic lesions were normally only single or double white necrotic rings round a green centre. These were slightly sunken, and if they occurred near the margin of the leaf, caused considerable malformation.

Perpetual or winter spinach (*Spinacea oleracea*). Chlorotic spots appeared on the inoculated leaves in about 6 days, which enlarged until the whole leaf was chlorotic and wilted. In 10 days the veins of the young leaves were cleared with some spotting or concentric chlorotic patterning. This was soon followed by severe veinal necrosis which eventually affected the whole plant.

On leaves which developed local lesions, the use of carborundum powder as an abrasive increased the number by five or six times. Keeping the plants in the dark for varying periods, both before and after inoculation, did not affect the amount of systemic chlorosis, but sometimes led to the formation of more individual rings.

The following test plants showed no symptoms when inoculated mechanically. Sap from the plants marked with an asterisk was inoculated back to tobacco without causing infection, so that they would seem not to be carriers: **Beta vulgaris* L., red and sugar, **Brassica sinensis* L., **B. oleracea* L. var. *capitata* L., **Calendula officinalis* L., *Cucurbito pepo* L., **Dahlia variabilis* L., *Hyoscyamus niger* L., *Lycium barbarum* L., **Medicago lupulina* L., **Melilotus officinalis* Willd., **Mimulus luteus* L., **Phaseolus vulgaris* L., var. Canadian Wonder, **Phlox drummondii* Hook, **Pisum sativum* L., vars. Alderman, English Wonder, Lincoln, *Silybum marianum* Gaertn., **Solanum capsicastrum* L., *S. capsicum* L., *Tropaeolum majus* L. and **Vicia faba* L.

DISCUSSION

Although the symptom picture caused by this virus on tobacco is a ringspot type, the host range and physical properties are not similar to any previously described ringspot virus, and on red currant it bears no resemblance to reported virus diseases of that host. Also cross-immunity tests with tobacco ringspot virus show no relationship, so it must be considered a separate virus.

My sincere thanks are due to Dr Kenneth M. Smith for helpful criticism, and to the East Malling Research Station for a supply of red currant seedlings.

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Fig. 1

Fig. 2

Fig. 3

Fig. 4

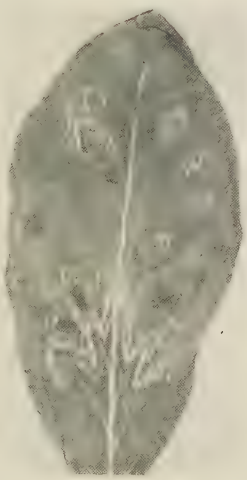

Fig. 5

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EXPLANATION OF PLATE 21

Fig. 1. Red currant seedling. Artificial infection. Young leaf shows chlorotic spots, next with chlorotic ringspots and resulting puckered growth, and old leaves with chlorotic flecks or rings.

Fig. 2. *Petunia* plant. The older leaves show chlorotic patterning and malformation, and the young leaves are extremely chlorotic, with only the tips a normal green.

Fig. 3. Tomato. Leaves showing an intensive chlorotic ring and line patterning or ringspots.

Fig. 4. Tobacco. Young leaf showing winter symptoms of diffuse chlorotic mottle.

Fig. 5. Tobacco. A mature leaf with vivid chlorotic patterns usually circular but tending to elongate along the veins, and the oak-leaf pattern at the base. ($\times \frac{1}{2}$.)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Ordinary Meeting of the Association held on Wednesday, 13 December 1950, in the Imperial College of Science and Technology, London; the President, Mr G. Fox Wilson, in the Chair.

After the formal business, the following papers were read and discussed:

Some aspects of applied biology overseas

1. Plant pathology in the Colonies. By Dr S. P. Wiltshire.
2. Some weed problems in the Sudan. By Dr W. F. Andrews.
3. Cacao pod diseases in West Africa. By Mr H. Owen.
4. Control and administration of entomological research work in the Colonies. By Dr W. J. Hall.
5. The work of the colonial government entomologist. By Dr W. F. Jepson.
6. The entomological problems associated with swollen shoot virus disease of cacao in West Africa. By Mr A. H. Strickland.
7. Development of insecticide-resistant fly populations. By Mr J. F. Newman.

Annual General Meeting of the Association held on Friday, 9 February 1951, in the Imperial College of Science and Technology, London; the Vice-President, Mr W. C. Moore, in the Chair.

After the formal business of the Meeting the following scientific films were shown:

1. Récherches expérimentales sur les champignons prédateurs des nematodes du sol.
2. The medical aspects of venomous snakes.
3. Insect pests on food.
4. Improving raspberry and strawberry crops.

Ordinary Meeting of the Association held on Friday, 16 March 1951, in the Imperial College of Science and Technology; the President, Mr R. W. Marsh, in the Chair.

After formal business, the following papers were read and discussed:

1. Observations on the wood-mouse (*Apodemus sylvaticus*). By Mr A. Roebuck.
2. Rats, mice and agriculture. By Dr D. Chitty.
3. Rabbits and other rodents. By Mr H. V. Thompson.
4. Birds in relation to agriculture. By Dr J. W. Evans.

In addition, two films were shown: 'The rural rat' and 'The rabbit pest'.

CACAO POD DISEASES IN WEST AFRICA

By Harold Owen, *University of Reading*

This paper is based on a relatively short experience of cacao in West Africa, and on some simple experimental work which I undertook while working at the West African Cacao Research Institute, at Tafo in the Gold Coast. The problems with which I am going to deal briefly may, I feel, be of some general interest, in that they illustrate how the application of rational disease control measures may be frustrated by a variety of local conditions; in this case, particularly by the fact of the local agriculture being on an unorganized, small farm, peasant basis.

The title 'Cacao pod diseases...' is a little misleading, in that only one fungus disease of cacao pods is of any importance in West Africa. This is 'black pod' disease, caused by *Phytophthora palmivora*. Several other fungi occur very commonly on cacao pods, but they are almost entirely secondary organisms, following damage of one kind or another. *Botryodiplodia theobromae*, ubiquitous in the tropics, is the principal one of these saprophytic or weakly parasitic fungi.

Next to black pod disease, most damage to cacao pods is done by squirrels, partly directly and partly through their wounding the pods and allowing the entrance of fungi.

Various insect borers cause widespread damage, but this is largely restricted to the husk; while capsid bugs, which damage the majority of pods, normally cause only superficial damage.

The following figures show the relative importance of these major types of damage. They were taken from observation plots of ordinary mature cacao at Tafo; the total area was 1 acre, made up of eight scattered plots, comprising a total of 520 trees, and the figures come from a complete year's harvests:

Disease or pest	Pods attacked (%)	Pods discarded (%)
Black pod	14·5	9·7
Squirrels	7·4	5·2
Borers	21·3	1·4
Capsids	78·6	0·4
Total for other factors		0·7
Total percentage discarded		17·4

Black pod disease, therefore, was responsible for the loss of over half the pods which were discarded; nearly 10% of the total crop was destroyed by this disease alone.

The estimate of the crop loss provided by the above figures is a conservative one, so far as West Africa as a whole is concerned. The season in question (1949–50) was admittedly one in which weather conditions were rather favourable to the development of black pod disease. But against this must be set the fact that the harvesting procedure was such as to minimize the losses: under native practice the loss of crop would have been much greater. In the observation plots harvesting was very frequent, which exerts a marked control over the disease, as I shall try to explain a little later. Probably the average annual loss of crop from black pod disease over the entire cacao-growing territories of West Africa is of the order of 20%.

Influence of rainfall upon incidence

The incidence of black pod disease is influenced considerably by the weather, and particularly by the rainfall. Dade (1931) showed that infection by *Phytophthora palmivora* takes place only when the pods are covered with a film of moisture, and atmospheric humidity is the key factor of the environment. Temperature is not a critical factor.

Atmospheric humidity is, of course, bound up with rainfall, and there is a more or less close correlation between the rainfall during the early part of the cropping season and the amount of infection by *P. palmivora*. Much the greater part of the cacao crop in West Africa matures between September and January, the peak harvest period being about November. Heavy rainfall in the earlier part of the season causes a high incidence of black pod disease on the young developing pods, so that there are abundant sources of infection present, even if the weather later becomes rather drier and therefore less favourable to the spread of the disease. If, on the other hand, the earlier months are free from excessive rainfall, there is little build-up of the disease on the young pods, and a wet spell later in the season does not do very much damage, largely because relatively few foci of infection are present.

The critical months vary somewhat according to locality. At Ibadan in Nigeria, for example, records taken over 7 years indicate a very close correlation between the rainfall in August and the proportion of pods infected with *P. palmivora* in the main crop. At Tafo, Gold Coast, we have 11 years' records, only of pods discarded from all causes, of which something over half would be discarded due to black pod disease alone; there is, however, a fairly close correlation between percentage of discarded pods and total rainfall over the months July, August and September; if we consider only the last 5 years, for which period the harvesting system has been standardized, the correlation is very much closer.

Control measures

The early work of Dade (1926, 1931) on black pod disease in the Gold Coast seemed to show that effective control of the disease by applications of fungicides was not possible and certainly could not be economically practicable. 'Sound cultivation and sanitation' were recommended as control measures against the disease.

The low cocoa prices obtaining until a few years ago, and more especially the increasing destruction caused by the virulent swollen shoot disease, caused attention to be withdrawn from the problem of pod disease. Recently, largely as a result of considerable increases in the price paid for raw cocoa, interest once again focused on the considerable losses resulting from the ravages of *P. palmivora*, and a programme of research into pod diseases was initiated at the West African Cacao Research Institute about 3 years ago.

One of the experiments carried out as part of this programme was designed to re-examine the possibility of controlling black pod disease by means of fungicides, and to obtain also an estimate of the degree of control possible by sanitation methods: by a harvesting system which would aim at the rapid elimination of foci of infection.

The experiment, on a randomized block layout covering about an acre, involved four treatments: first, spraying with Bordeaux mixture; secondly, spraying with the proprietary copper oxide fungicide Perenox; thirdly, weekly harvesting; fourthly, control. The plots under the spraying treatments, and the controls, were harvested every 6 weeks, which is a little more frequently than the general practice among the farmers. At the harvests, all ripe pods and all diseased pods were picked.

The fungicides were applied every week, by knapsack machines. At this frequency, of course, spraying could never be an economic procedure. But it was considered best, in the first instance, to establish whether or not black pod disease could be controlled by chemical methods under tropical rain forest conditions, and to consider later the economics of the subject, and the optimum interval to be adopted between spray applications, if the preliminary results were promising.

The records of the 1949–50 season showed that a considerable control of infection by *P. palmivora* was obtained by fungicide spraying, but there was little diminution in the incidence of black pod disease in the weekly harvested plots compared with the controls, though the difference was statistically significant:

Treatment	Mean percentage of pods infected with *P. palmivora*
Control	14·6
Weekly harvesting	11·5
Perenox	2·7
Bordeaux	1·9
(Critical difference	1·9)

However, the percentage of infected pods does not give a true valuation of the efficiency of a control measure. An infected pod is not necessarily worthless, and may give a full yield of good beans, if it is fairly mature. The husk of a cacao pod is quite thick and succulent, and a period of the order of 3 weeks usually elapses before the fungus progresses into the interior of a pod and causes perceptible damage to the contents, although if a young pod is attacked the beans do not mature in any event.

The rationale of frequent harvesting is, first, to pick the more or less ripe pods before the contents of infected ones have become invaded by the fungus; secondly, to eliminate infected pods before the fungus has produced its sporangia, and so has become a source of infection. (Sporangia are produced about a week after infection has taken place.)

Hence a more useful criterion on which to base a judgement of the relative efficiencies of the several treatments is provided by the average weight of usable beans obtained from the pods:

Treatment	Mean weight of good wet beans per pod in oz.
Weekly harvesting	3·81
Perenox	3·58
Bordeaux	3·50
Control	3·32
(Critical difference	0·25)

(Percentage increase, weekly/control = 14·75)

The above table shows that weekly harvesting gave the greatest mean weight of good beans per pod: significantly greater, not only than the control figure, but even than that from the plots sprayed with Bordeaux mixture; yet spraying with Bordeaux mixture resulted in the lowest incidence of black pod disease.

The superiority of weekly harvesting over other treatments, with respect to mean weight of good beans per pod (which is, effectively, total yield of marketable product), is of great interest. It shows that the control measure which has for many years been recommended against black pod disease does give results. The increase in yield of beans over the control treatment was nearly 15%.

It seems very unlikely that spraying with fungicides, even at intervals considerably greater than 1 week, could stand competition, financially speaking, with the simple measure of picking the pods at very frequent intervals. Chemical control would, moreover, involve very much more trouble in every way; apart from the cost and upkeep of machinery and the cost of the chemicals themselves, obstacles to the successful introduction of fungicide spraying under West African conditions are, for example, the difficulty of mixing and application with unskilled and illiterate labour, the difficulty of applying sprays due to irregular planting, and,

probably of the greatest significance, the general unavailability of adequate supplies of clean water for mixing spray fluids. So far as the last point is concerned, fungicidal dusts might offer a solution, but their adhesive capacity when subject to violent tropical rain would almost certainly be inadequate.

Discussion and conclusion

To reduce the losses caused by black pod disease, then, frequent harvesting, at intervals of about a week, would appear to be a very worthwhile measure, especially when there is excessive rainfall during the early part of the season. Unfortunately, there seems little likelihood of such a procedure being widely adopted by the cacao-growing peasant community of West Africa. An immediate objection from the farmers is that their labour requirements are increased. But a much more fundamental obstacle is that resulting from the small size of the cacao farms, and from the necessity of fermenting cacao beans before they are acceptable to the market.

This fermentation process, in which the beans are massed together for a few days in heaps or boxes, is necessary, first to remove the mucilage which surrounds the beans when they are taken from the pod, and secondly to cause a change in the chemical constitution of the bean itself, in the absence of which change the characteristic chocolate flavour cannot be developed in the manufacturing process which comes later. To produce the required temperature for the fermentation to proceed satisfactorily, a considerable mass of beans is necessary. Now the cacao farms of the Gold Coast and Nigeria are very small (2 or 3 acres on average), and if a farmer with only that small area of cacao trees picks his pods every week he cannot obtain a large enough quantity of beans at each picking to ferment satisfactorily. Thus it is that the usual practice is for farmers to harvest the crop only two or three times during the whole season, at intervals of 2 months or more, with consequently very high losses due to black pod disease, which is always present to a greater or lesser extent, and under favourable conditions is able to spread through the crop virtually unchecked. (In Nigeria the disease appears to be more destructive than in the Gold Coast, and this may largely be accounted for by the fact that many of the cacao farms in Nigeria are even smaller than those of the Gold Coast.)

Under the prevailing conditions of land-tenure and land-utilization it would seem that great benefit could be derived from the adoption of some communal system. In the West Indies, co-operative fermentaries have been in existence for a number of years and work very well. An attempt was once made to establish such an enterprise in the Gold Coast, but it met with little success. The West African is apt to be very suspicious; corrupt practices are so widespread that a farmer is most reluctant to put his beans into a common pool before he has been paid for them (and full payment could not be made prior to fermentation). There is a shortage of European staff and none can be spared to initiate a co-operative scheme and see it through its early stages.

So it is that there seems little hope, in the near future, for any reduction in the loss of cacao crop in West Africa from pod diseases. Yet there seems little doubt that, if the benefits to be obtained by frequent harvesting were fully appreciated by the majority of the farmers, and such obstacles as exist overcome, as they could be, cacao production could be greatly increased.

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THE WORK OF THE COLONIAL GOVERNMENT ENTOMOLOGIST

By W. F. Jepson, *Imperial College Field Station, Silwood Park, Sunninghill, Berks*

(With 2 Text-figures)

We have heard from Dr Hall evidence of the tremendous impetus which is being given, both by local colonial governments and by the Home authorities, to the more fundamental investigation of insect outbreak in the colonies. And there is none to whom this development is more welcome than the colonial government entomologist.

I have for the last 2 years been able to contemplate, from the point of vantage of a university institution, the pros and cons of a system in which I worked happily for 15 years. I hope I shall be able to give you a picture of the status and scope of the government entomologist, with particular emphasis on the non-self-governing colonies.

First, how does the scientific specialist fit into the scheme of government? The senior entomologist is, in fact, the only man on the spot whom the Government can consult whenever and wherever insect problems arise. Whether it be bees in the roof of the Governor's study or a calamitous locust outbreak, the matter is referred in the first instance to the entomologist, and he is expected to do something about it.

As an example, I have drawn up a 'family tree' (Fig. 1) showing the relation of the entomologist to the other officers in a typical agricultural department.

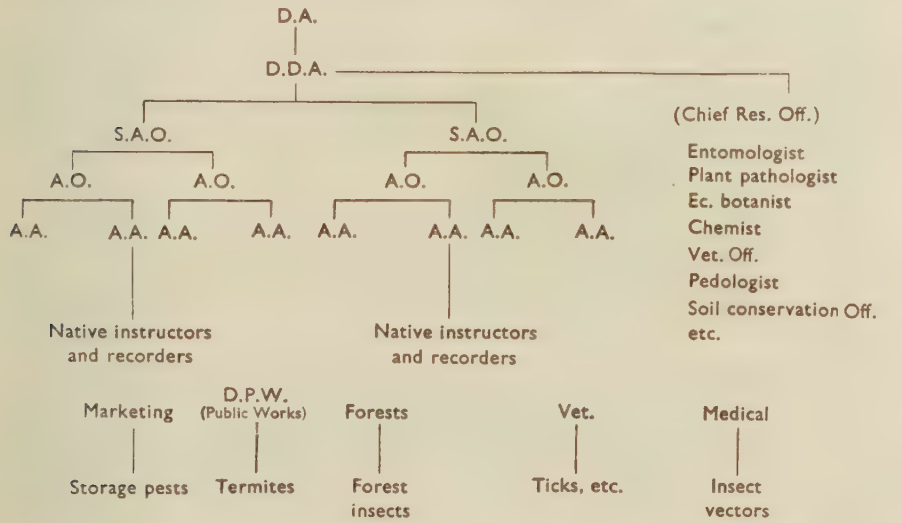

Fig. 1. Existing administrative relationships in a typical colonial agricultural department. D.A. Director of Agriculture; S.A.O. Senior Agricultural Officer; A.O. Agricultural Officer; A.A. Agricultural Assistant.

You will observe that the specialists are not as a rule in the direct line of hierarchy. Although in the larger colonies there is sometimes a chief research officer between the D.D.A. and the specialist, the work of the entomologist is usually controlled by an agriculturist or medical man. It is perhaps an old tradition that the scientist should be on tap not on top. Without labouring the point, I would express the opinion that such a system tends to lead to the frustration of the individual and to deprive the specialist of vigorous scientific direction and of co-ordination of his work with that of his colleagues.

The entomologist, however, has little time to dwell on such morbid thoughts; when he is not inundated with the routine administration of his little autonomous section, he has to meet the demands, not only of all the officers in his own department, but those of a number of others who may not have their own entomologist.

The medical entomologist may, for example, be a taxonomist in the Culicinae, and will not have the insecticides, equipment or experience to deal with a widespread outbreak of ticks or houseflies in a remote part of the country.

We must, however, look back for a moment, over the history of colonial entomology to the days of the first World War. Confronted with a strange and extensive fauna, the first task of our entomologists was to collect and classify the insects associated with economic crops, whether they were actual pests or not.

We all know the pioneering work done in this field by Dr Sheffield Neave, Sir Guy Marshall, Dr Hall and many others.

In this work, the Imperial Institute of Entomology with its publications has been the nerve system and indeed life blood of colonial entomology.

The next phase was the field work on ecology and cultural and biological control measures, typical of the period including the economic depression of the early thirties. Outstanding in this period were the successful control of coconut insects in Fiji by Dr Taylor and his co-workers. It was also during this period that many entomologists, frustrated by the inevitable economies, were unable to implement control schemes, and took refuge in the study of special groups of insects. I think that there are many advantages in a government entomologist becoming a specialist in a particular group. We are all fully conscious of the cardinal importance of sound taxonomy in Applied Entomology in newly developed countries. On the other hand, the man whose spare time is otherwise occupied, be it with bebop or native local lore, may gain enormously in the field of human relations. In my experience the man who specializes too narrowly is not always appreciated either by the administrator or by the planter. One of the first duties of the government entomologist is to enlist the willing co-operation of colleagues, planters and the local peasant, in his pursuit of the data he must collect if he is to acquire a masterly grasp of the pest situation.

The scope of the government entomologist has been greatly enlarged by wartime developments. Most of us were pressed into service on food protection, locust, or malaria control, and with the advent of DDT and Gammexane, we were able to implement many of the ideas which were previously tucked away as impracticable. Since the war, we have been constantly reviewing our list of major insect pests, to see how the control picture may have been altered by the advent of the new synthetics and by new mechanized and grouped agricultural practices.

Considering my own particular share of advisory work in Tanganyika, with 28 major problems, you may care to tell me how the government entomologist can get very far in research work. Time will only permit me to pick out one salient example, the stalk borers.

I do this because there has been a tendency in the past to avoid grappling with pests of native food crops. Generalized as a world problem, the moth borers in graminaceous crops—cereals and sugar cane—are, in my opinion, commensurate in importance with locusts.

Acting surreptitiously in every field in every year, stalk borers rob the peasant of some 2–5 % of his subsistence crop, and the sugar planter of an even larger part of his yield. Here I feel is a chance for concerted action in many of the African colonies; in India, Africa, China and America. We have a great mass of facts about the ecology of the relatively few Noctuids and Pyralids involved.

What we need are well-planned field techniques and the trained technologists to implement them. Here the government entomologist has welcomed the recent great efforts of industrial firms in sending their scientific men to find out at first hand what formulations and equipment are appropriate to his locality. The entomologist, working in remote areas, finds these visits invaluable in that he learns something of current programmes of work in other areas. I found

myself much in favour of a system whereby the clinical entomologist, as Sir John Fryer used to describe him, does the diagnosis and writes a prescription which the technical operator will, in the near future, be in a far better position to carry out, than is the entomologist himself.

I think such a system will gradually evolve, at least for plantation crops.

A valuable and long overdue addition to our entomological forces has been the arrival of teams working under the Colonial Development and Welfare schemes, the Empire Cotton-Growing Corporation and industrial firms on special problems. We have the Colonial Insecticide Unit in Tanganyika and there are teams working on stored products, Tsetse fly and cacao in West Africa. Whilst the government entomologist is usually aware of the work of these teams, such liaison is very often unofficial, which is normal and proper from the purely scientific viewpoint. The feeling of the government entomologist is that the co-ordination of the programmes and results of all entomologists working within the country or group of neighbouring countries should be strengthened. One way of doing this would be to allow the specialist more weight in framing local scientific programmes and policies.

Harking back to the departmental family tree, Fig. 2—not intended to be an impertinent piece of advice—but a hope, for the larger colonies, shows one way in which this could be achieved.

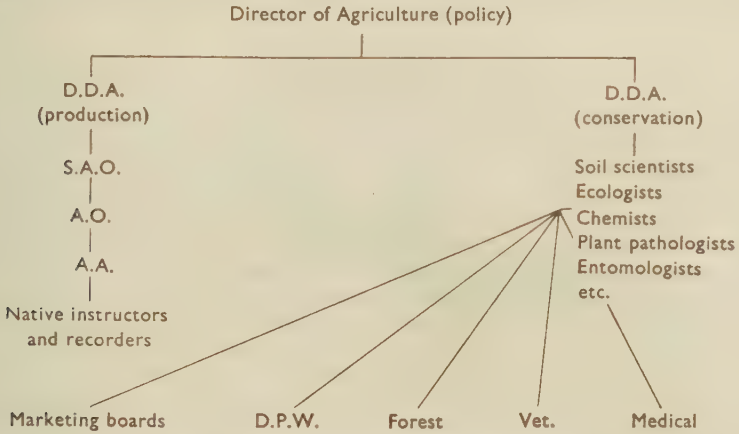

Fig. 2. Proposed administrative relationships.

The scientific specialist should be regularly eligible to sit at the level of deputy director of his department, in direct charge of all scientific and technological investigation separable from routine or extension work. This Deputy Director should be required to know what is going on in neighbouring territories and should be in close contact with specialist research institutes in the United Kingdom and elsewhere. The knowledge that he belongs to a professional scientific unit, directed by one competent to assess the value of his scientific work, will enable the government entomologist to contribute more effectively to the progress of his territory.

DEVELOPMENT OF INSECTICIDE-RESISTANT FLY POPULATIONS

By J. F. Newman, *Jealott's Hill Research Station, Bracknell, Berks*

The paper described recent work in Sardinia on the problem of controlling flies that had developed resistance to various insecticides. This work was carried out in collaboration with the Rockefeller Foundation who, in their successful operations to control malaria in Sardinia, had reported that house-flies had become highly resistant to DDT and to Chlordane.

The author carried out field trials with various benzene hexachloride formulations and found that neither dispersible powders nor emulsions controlled these resistant flies. BHC had been used extensively on the island for locust control, and it is possible that this had aided the development of strains resistant to this insecticide. Strains of non-resistant flies imported from Rome were completely susceptible to all three insecticides.

OBSERVATIONS ON THE WOOD-MOUSE (*APODEMUS SYLVATICUS*)

By A. Roebuck, *National Agricultural Advisory Service, Shardlow Hall, near Derby*

The animal

It is a little larger than the house-mouse with a tail as long as its body and is often called the long-tailed field-mouse. The upper parts are brown or yellowish brown, and the underparts, including the feet, white. It has big ears and large staring eyes and is nocturnal. It is a very active excitable creature ranging up to 200 yards from its burrow during the night. The wood-mouse is our most abundant mammal, occurring on all kinds of lands and at all altitudes. It seldom comes into houses or stacks but will enter open greenhouses and garden frames. It does not hibernate.

Its burrows and nest

The wood-mouse is an active burrower and uses extensive runways about 2 in. under the surface of the ground. It naturally uses burrows made by moles and voles and therefore it is not always possible to tell which animal has made a particular burrow. One runway was followed for about 30 yards in an almost straight line. This gave off numerous side passages. In a plantation occupied by wood-mice there are innumerable entrance holes and the tunnels join or cross one another producing a veritable maze of passages just under ground-level.

The nest is made of grass or moss or a mixture of these with hawthorn or other leaves in a hollowed-out chamber about 3 ft. below the surface of the ground. There are said to be three entrances: we have only found two but always with evidence of a third. From the entrance holes curving passages lead to the nesting chamber.

The burrows are found in woods and thickets, along hedgebanks and dike sides, but very rarely in open fields.

Feeding habits

The wood-mouse's food is principally vegetable, but it will eat almost anything vegetable or animal, and is frequently a cannibal in captivity.

We have had records of it eating sown peas, digging up and eating crocus bulbs (corms), entering garden frames and grazing carnation cuttings in addition to the extensive grazing of cereals.

Wood-mice have severely damaged young fruit trees by nibbling and girdling the bark. Much damage is also done in this way in woods especially during severe weather. This habit they share with other rodents.

They eat acorns, beech mast, nuts, corn, peas, bulbs, roots, haw seeds, etc.

During the summer we found they seemed to show a preference for beetle grubs, caterpillars and worms. So far as we could see they would not eat grass, at any rate in the summer period.

Bell's *British Quadrupeds* states: 'The devastations committed by it are almost incalculable.'

With us it has eaten wheat seedlings in three ways:

(i) Nibbling off the young shoot as it appears above ground. This is identical with rabbit damage.

(ii) Scuffling up the young plants and eating some of the seed and some of the tops, but scattering the rest about the ground.

(iii) Eating out the heart of the young plants. This habit is shared by the field vole.

When the corn is ripe the wood-mouse will ascend the stems and eat some grains, at the same time scattering more. It collects heaps of grain under the stooks at harvest time.

It shares with the field vole the habit of gnawing through the stems of cereals to fell them in order to get at the ears. It does this with linseed also.

Sometimes it makes extensive stores of food but doesn't seem to use them.

Reproduction

Sandars, in his *Beast Book for the Pocket*, writes: 'litters of four to seven, sometimes five times a year. Breed continuously all summer. Period of gestation is 3 weeks. Females breed at 5 months old.'

It lives about 18 months.

Prof. Ritchie in his book, *Beasts and Birds as Farm Pests*, writes: 'In grass-lined nests the young, averaging about five a litter, are born. The breeding period continues over the greater part of the year; and this together with the fact that litters follow each other at intervals of rather less than 4 weeks, accounts for the rapid multiplication of the species in suitable years, although widespread plagues comparable to those of the field vole are unknown.'

Barrington, in the *Zoologist* of 1881, gave an account of breeding the wood-mouse in captivity. We have so far not succeeded, although it seems to be quite happy in captivity.

Numbers and movements

We have studied it in plantations and woods. We have tried it in the laboratory and find it a most difficult creature for such work, and also for small plot work. It seems to be impossible to determine the population of a wood.

Wood-mice can readily be trapped alive or dead. They do not, however, enter the traps during a period in the summer. The numbers trapped vary considerably from year to year.

We have studied the movements by releasing marked animals by clipping marks on the fur and by snipping the ears. One was recaptured 200 yards away from the point of release, in the next field. We tried ringing by plastic and metal rings (Chitty, 1937) as used for small birds, but found them most unsatisfactory as they spent most of their time indoors either gnawing off the rings or even sometimes their feet.

Elton and collaborators studied particularly the *Apodemus* population of a wood from 1925 to 1928. They found 1926 a year of peak population and a big fall in numbers in 1927. They say, 'It was found that the changes in numbers of *Apodemus* were different from those known to occur in *Microtus* but a mortality occurred in *Apodemus* near the time predicted.... It is not possible to say definitely whether or not there was an epidemic.'

The problem in the East Midlands

In 1934 crocus corms were taken by wood-mice in large numbers. A hole was made against the plant and the corm removed. The tops of the plants were either left standing as if untouched or were lying on the ground near the holes. Peas were extensively taken in gardens and market gardens. Carnations in frames were grazed. Wheat in two localities was suspected of being grazed by mice.

In 1938–9 winter wheat was again grazed on the old sites and there were one or two more suspicious cases.

During the winters of 1941, 1942 and 1943 wheat grazing was studied at Legsby (Lincs) after rabbits had been cleared as much as possible. The grazing proceeded in a straight line parallel with the wood. This we termed the feeding front. The maximum rate of advance was 5 yards a night. Wood-mice were trapped in abundance along the feeding front. We trapped the mice alive and got them to snip wheat in the same way as on the field. This was not easy owing to their burrowing activities in their efforts to escape. We then, after consultation with the Bureau of Animal Population at Oxford, used the pre-baiting method and final poisoning with zinc phosphide. Crushed oats was placed on trays protected from other animals and

birds but allowing ingress of the mice. After a few nights it was poisoned by the addition of 4% zinc phosphide. The attack at once stopped. Traps were laid regularly in the field. After about 3 weeks mice began to appear again. Repetition of the pre-baiting and poison baiting caused the attack to stop.

In 1943–4 we treated other fields in different localities with the same result. We never got the attacks more than 165 yards from the wood, and the attack would not cross a dike with water in. We concluded that wood-mice were responsible for this type of grazing. Rabbits, however, do graze it in the same way and probably so will *Microtus*. We thought at the time we could tell the bite of the wood-mouse from that of the rabbit, but subsequently in the laboratory this proved to be wrong. This means that our only guide is a regular advancing line which can be stopped by the method of pre-baiting and poison baiting.

During the winter 1946–7 in a Nottinghamshire area being studied a field of beans was attacked (on a previous wood-mouse-grazed field) like the crocuses. The beans were removed to old rabbit burrows and stored in large quantities. These were covered with soil and wood-mice trapped from exit holes which they made. Subsequently this carrying beans to store was observed in the laboratory.

In 1948–9 some wheat was slightly grazed. Beans were scarcely touched. Trapping gave fewer mice. There has been a rapid fall-off in trap catches from then to the present day.

A review over the whole period suggests there may be big population changes and grazing of winter cereals occurs noticeably in periods of abundance.

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RATS, MICE AND AGRICULTURE

By Dennis Chitty, *Bureau of Animal Population, University of Oxford*

Most of the recent work on the biology and control of the brown rat and house-mouse is still unpublished, and I am using information collected by Mr Elton, Mr Middleton and others of my colleagues who worked on rats at the Bureau of Animal Population during the war. I am also using data for the house-mouse published by Mr Southern and Miss Laurie in 1946.

Rats occur in fields, no doubt in most parts of Great Britain. The damage they do there is probably rather small, but fields are important as a reservoir from which the farm buildings and ricks may be invaded in the autumn. Damage to sacked goods in buildings is often a spectacular sight and may stir the farmer to prevent it. Losses from bins or troughs is much less obvious and may not be fully appreciated. A small example of an insidious loss of this kind was found in 1940. A flock of 120 hens was supplied with a continual surplus of dry mash which rats were known to be sharing. Measurements of the amounts taken showed that the rats were actually eating more than the hens were, at a cost to the farmer of about £1. 10*s*. 0*d*. per month.

Ricks are mostly built about August and threshed at various times up to early April, though some stand until later. Rats start entering the ricks soon after harvest and some may have maximum infestations of about 150 rats in January. Considering all ricks, however, the average density of rats increases throughout the first 3 months of the year. There is continual breeding and it may be the surplus of young that accounts for the belief that rats leave the ricks in spring. In any case plenty remain, however late the date of threshing.

In the Oxford district only 6% of ricks had no house-mice in them by April; almost half had fifty or more mice by then and densities up to fifteen per cu.m. were found.

The amount of wheat eaten in 3 months by rats and mice is estimated at over 1400 tons for every 100,000 ricks standing on 1 January. This estimate allows for the rate at which ricks are threshed but not for waste of grain in addition to that eaten.

A technique of rat control by pre-baiting and poisoning was thoroughly investigated and proved to work well in nearly all environments. The necessity for pre-baiting (or some equivalent technique) was clear once the behaviour of the rat had been studied. Rats are suspicious of unfamiliar objects or foods and are also unlikely to eat freely if they encounter a bait after they have filled their stomach at their accustomed time and place. Quite simple baits were successful, even in ricks. Here, however, there is a possible danger from poison residues and, unless the operators are very reliable, poison should be placed only in holes in the ground underneath. A universally safe method of rat control in ricks has not yet been tested.

A large-scale test of the practicability of the methods recommended for rat control was carried out on 11,500 acres of farmland in Berkshire. In the 3 years before the scheme started the average numbers of rats killed per rick at threshing were 27, 17 and 23 (in 1939–42). In the next 3 years the numbers killed at threshing were 3·5, 1·2 and 1·2 per rick. These ricks, however, were baited in holes in the sides, not in ground holes only. In all other habitats, equally good control was achieved, and it was estimated that one man working full time could treat 25,000 acres at an annual cost (in 1946) of 2*d.*–3*d.* per acre.

There is no doubt that the losses to rural rats and mice are great. It has also been found that most of this loss can be prevented given high enough standards among the county rat poisoners. An interesting human problem arises, however, where the rats have been so reduced that further results are not sufficiently spectacular to retain the interest of the operator. Maintenance is tedious and costly in comparison with the numbers of rats killed. Cats, however, though unable to deal with well-established infestations, have proved admirable at preventing serious re-infestation after successful treatments with pre-baiting and poisoning.

RABBITS AND OTHER RODENTS

By Harry V. Thompson, *Ministry of Agriculture Infestation Control Division, Tolworth, Surbiton, Surrey*

Rabbits

(1) *History*

Fossil rabbits are found in English Pleistocene deposits but the species probably disappeared in the succeeding glacial epoch. It is not mentioned historically until the twelfth century when it was probably introduced by the Normans. It was abundant in England about the fifteenth century and although present in Scotland, was little known there until the nineteenth. It was highly valued for its food and fur until this century, during which its destructiveness has become more widely appreciated.

(2) *Place of rabbits in the rural economy*

There has in the past been much dispute about the value of rabbits to agriculture and well-meaning persons have introduced them into various parts of the world (e.g. England in the twelfth century, Australia and New Zealand in the mid-nineteenth and Chile in the early twentieth century). Most countrymen are partial to a rabbit for the pot and sporting and pecuniary interests ensure that the rabbit does not lack advocates. However, most competent farmers are agreed that the rabbit is an unmitigated pest.

Since large animals require less energy per kilogram of body weight for maintenance than do small ones, it is a biological truism that small animals require more food per kilogram of

body weight than large ones. Recent nutritional work has also shown that pasture digestion in sheep and cattle is more efficient than in the rabbit. Figures for basal metabolism show that six doe rabbits require about as much useful energy as a ewe for maintenance. The live dressed weight ratio favours stock as compared with the rabbit, and the method of harvesting beef and mutton is controlled and much more efficient than the harvesting of rabbit meat.

While the complete extermination of rabbits is a pipe dream, only minimum numbers can be tolerated by a healthy agriculture.

(3) *Damage*

(*a*) *Pasture*

It may be considered that rabbits grazing near their burrows do not do a great deal of damage, particularly in hill grazings, but the total effect of a number of colonies is clearly considerable (Fenton, 1940). Damage to pasture is less obvious than that to arable crops, although disturbance of the soil surface by rabbits assists in the establishment of the conspicuous ragwort *Senecio jacobaea* (Cameron, 1935).

An interesting study has recently been made by Phillips (unpublished) in Cardiganshire. A pasture was directly reseeded with a mixture of perennial and Italian rye grass, red and white clover and rape. Two 1-acre plots were fenced with rabbit-proof wire and two with pig-netting wire. The herbage was controlled by lambs for a period in the autumn of the sowing year and in the following spring.

The general effect of rabbit grazing on the pasture was to cause a marked reduction in the yield of total herbage (as determined by sample cuts and plant counts) particularly of the sown species, while there was an increase in the yield of weeds. The rate of growth of the herbage and the feeding value of the pasture in terms of lamb live weight increment were also adversely affected.

Rye grasses were eaten by rabbits in preference to clover and rape.

An experiment similar to that of Phillips is being conducted at Wye Agricultural College, the main differences being that it is on permanent chalk grassland as compared with siliceous reseeded pasture, and that the emphasis is upon the number of sheep displaced by a population of rabbits rather than on herbage analysis.

An area of 6⅝ acres has been surrounded by a rabbit-proof guard fence, inside which is a rabbit warren of ¾ acre cut off by rabbit-proof netting from six plots of just under one acre each. From the warren the rabbits have access to three of the six plots through nine tunnels let into the netting.

Sheep are grazed for 2–3-week periods, followed by 2–3-week rests, through spring, summer and autumn, and herbage samples are cut from inside and outside rabbit-proof cages before and after each grazing period. It is thus possible to calculate the herbage eaten by the sheep on the rabbit-free plots, and that eaten by rabbits when alone and rabbits and sheep when together on the rabbit-grazed plots. Records are kept of the numbers of sheep maintained on the plots and increases in their weight. Botanical changes in the sward of each plot are being studied, and rabbits are periodically live-trapped, marked, weighed and watched through field glasses and a telescope. During the first 7 months of the experiment (May to November 1950) the sheep live-weight increase on the rabbit-free plots has been 800 lb. and on the rabbit-grazed plots 650 lb.

(*b*) *Arable crops*

Damage to market-garden crops, mangolds and sugar beet, kale, cabbage and lucerne are well known, and grazing of cereals is especially serious. Members of the National Agricultural Advisory Service are well aware of the importance of this damage, and the attention of some of them has been drawn particularly to the grazing of winter cereals (Gough & Dunnett, 1950). Entire fields of winter wheat or oats may be grazed down to the ground in a few weeks or even days, and the number of plants so reduced that resowing is necessary. Advisory entomologists of the N.A.A.S. are currently making further observations on this type of damage, to

determine its extent, the time of year at which it is most serious and also to demonstrate to farmers its importance.

While rabbit damage to spring cereals is less catastrophic, and more localized in its incidence, it may nevertheless seriously reduce the crop. A pilot survey of the damage to spring wheat in Kent has been undertaken.

(*c*) *Trees*

Rabbits are a source of great anxiety to foresters, preventing natural regeneration by eating seedlings, distorting the growth of trees by damaging leading shoots (Watt, 1919; Forestry Commissioners, 1943), and killing trees by ring-barking. Orchards are particularly vulnerable especially when pasture is scarce and, in a test of rabbit-repellent substances currently taking place at Wye, Kent (Thompson & Armour) 44 out of 44 control (untreated) apple rootstocks have been gnawed by rabbits within the first 2 months of the experiment.

(4) *Methods of control*

The established techniques of trapping, ferreting, gassing and snaring, with the use of dog and gun and the long net, should all be used, according to the terrain. The use of poison baits and viruses is not considered advisable in this country and the former is illegal. Research is being done on the comparative efficiency of the various methods of control in different environments.

The place of natural predators as a factor in rabbit control is far from being fully understood, and long-term observations are necessary. Experiments in some other countries, e.g. New Zealand, where thousands of ferrets, stoats and weasels were liberated in an effort to control rabbits, have not met with success.

As in the control of rats, it is essential that the clearance of rabbits from an area of adjoining farms and estates should be a co-operative enterprise in block control, so that re-infestation can be reduced to a minimum.

The cost of rabbit clearance is higher in areas of abundant cover, long-standing infestation and entrenched rabbit trapping industry. In such areas land improvement, mutual aid and constant vigilance on the part of owners and occupiers of the land, with resort to compulsory powers under the 1947 Agricultural Act when necessary, offer the only hope of control.

Other rodents

Reference was made to the damage done by indigenous species: hare, harvest mouse, bank-vole, dormouse, water-vole, red squirrel and field vole; and that attributable to recently introduced species; grey squirrel, coypu, musk rat, and edible dormouse.

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BIRDS IN RELATION TO AGRICULTURE

By J. W. Evans, *Mill Field, Mill Lane, Chalfont St Giles, Bucks.*

Short accounts were given of the reported harmful activities of the following birds: the wood-pigeon, rook, jackdaw, carrion crow, magpie, jay, blackbird and sparrow, and these were followed by an examination of some of the claims that have been made that birds are of value in assisting to control insect pests. An impression is widely held that, because most birds that damage crops also eat insects, the harm they cause to agriculture is to a large extent balanced by the benefits they confer. Statements are often made, for example, that rooks are a factor of significance in wireworm control, yet it is certain that the numbers of wireworms they may eat in a field, in relation to those actually present, must be so small as to be of negligible importance.

There are, nevertheless, some few undoubted instances where birds do play an important part in insect pest control; for example, it is well known that migrating storks in Africa frequently prevent the further development of incipient locust and Army Worm outbreaks. Instances of this nature are, however, of exceptional occurrence.

It was suggested that rather than seeking to protect birds on the unproven grounds of their usefulness to agriculture and horticulture, it would be more appropriate to call for their protection because they are delightful creatures, and as part of our heritage, to be encouraged in every way possible.

Man, like other animals, must feed, and in this country we have not got sufficient land on which to grow enough food to feed our population and we cannot really afford to pay to import all the food we buy abroad. Because of this, any creature that on occasion causes substantial damage to our crops should be prevented from so doing; if possible, by driving them off, and if this is impossible then they should be destroyed, not by methods of wholesale slaughter, but by reducing their numbers in areas where damage is being caused.

It was pointed out that for the control of insects one calls in an economic entomologist but that economic ornithology at present rests on so much assumption and approximation that it cannot claim to rank as an exact discipline of biological science. Nevertheless, much is already known about the control of birds, even though the knowledge supporting control measures is often of a slender nature. After particulars of control measures by the use of scarers, traps and narcotics had been given, a plea was made that birds should be considered on the same basis as other organisms that threaten crops and not placed in a special category for reasons based largely on sentiment.

In conclusion it was suggested that the only way in which the present position can be changed is by the establishment of a body of fact based on scientific investigation.

REPORT OF THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS FOR THE YEAR 1950

The Officers and Council of the Association were as follows:

President: G. Fox Wilson, N.D.H., F.R.E.S.
Vice-Presidents: W. C. Moore, M.A.; J. W. Evans, M.A., Sc.D.
Hon. Treasurer: H. F. Barnes, M.A., Ph.D.
Hon. Secretary: R. K. S. Wood, B.Sc., Ph.D.
Acting Hon. Secretary (from September): L. Broadbent, B.Sc., Ph.D.
Hon. Asst. Secretary: J. R. Busvine, D.Sc., Ph.D.
Hon. Editor of the *Annals of Applied Biology:* R. W. Marsh, M.A.
Hon. Asst. Editor: I. Thomas, M.Sc., Ph.D.
Council: J. R. Booer, B.Sc., Ph.D.; C. Ellenby, D.Sc.; J. W. Evans, M.A., Sc.D.; C. E. Foister, B.Sc., Ph.D.; Miss M. T. Franklin, B.Sc., Ph.D.; S. D. Garrett, M.A., Sc.D.; H. H. Glasscock, M.Sc.; H. C. Gough, B.Sc., Ph.D.; R. V. Harris, D.Sc.; F. H. Jacob, M.Sc.; A. B. P. Page, D.Sc.; Mrs M. A. Watson, B.Sc., Ph.D.

During the immediate post-war years (1946–9) the yearly average of fifty-five new members was abnormally high; during 1950 thirty-six new members were elected and one former member rejoined. In accordance with a recent ruling of Council the names and addresses of new members are not given in this report: they will, however, be included in the new list of members to be published at the end of 1951. The following sixteen resignations were accepted during the year; F. S. Bodenheimer, Mrs J. R. Catford, J. B. Duggan, Sir Ronald Hatton, Mrs D. T. Hinchcliffe, H. Jacks, E. W. Jones, H. W. Kerr, F. J. Meggitt, Miss R. M. Phillips, N. Polunin, A. P. Preston, J. Henderson Smith, Miss R. I. Stoker, Mrs M. B. Tuke and S. M. Whitteridge. On 31 December 1950 membership stood at 576, but the following twenty-five members resigned from the end of the year: F. H. Braybrook, Mrs E. L. Campbell (née Britton), H. Burman, S. Burr, R. N. Chrystal, Mrs E. H. Cooke, F. M. Dyke, Miss M. P. English, D. J. Finney, Mrs P. J. Maycock (née Ford), H. E. Harbour, J. Hunter-Smith, T. J. Jenkin, N. H. Pizer and R. Robson. The following six members are presumed to have resigned: Miss M. Addison, Chester Cooke, J. Harrison, D. J. Macleod, J. Ramsbottom and Miss J. P. Smith-Scott.

Honorary Membership of the Association was conferred at the end of 1949 upon Dr I. Trägårdh and during 1950 upon Dr E. C. Stakman and Mr C. T. Gimingham.

The Council and Association learned with deep regret of the serious illness of the President early in December and of his death on 9 January 1951.

The Council regrets to record also, the deaths during the year of the following two Honorary Members and three Ordinary Members: Dr L. O. Howard (Hon. Member 1905), the distinguished U.S.A. entomologist; Prof. N. H. Nilsson-Ehle (Hon. Member 1923), the distinguished Swedish botanist; Mr F. J. Chittenden (1908), one of the Association's oldest members; Mr L. R. Johnson (1938); and Mr H. Walkden (1923).

Seven meetings were held in London, with an average attendance of forty-eight members and twenty-six visitors. The following papers were read:

20 January. Symposium on the locust problem. B. P. Uvarov: Principles, policy and organization. D. L. Gunn: Research and development.

24 March. N. F. Flentje: Some aspects of Australian plant pathology. P. S. Nutman: The interaction of strain and host genetic factors in symbiotic nitrogen fixation. J. L. Harper:

The root surface flora of banana varieties and disease resistance. A. H. BUNTING: Technical aspects of the East African Groundnuts Organization. M. H. MOORE: The use of apple rootstocks in outdoor testing of fungicides. G. A. SALT: The effect of date of application of nitrogen to wheat in presence and absence of eyespot. A. V. DELAP: Striking effect of mineral nutrition on the ecology of a plant community. J. H. WARCUP: The soil plate method—a simple way of isolating fungi from soil.

21 April. Symposium on marine biology. W. C. HODGSON: Forecasting the herring fishery. I. D. RICHARDSON: Echo-sounding and pelagic fishes. E. W. KNIGHT JONES: The Essex oyster fisheries. P. R. WALNE: Oyster pests. H. A. COLE: Oyster culture at Conway.

29 September. A. C. EVANS: Entomological problems facing the Overseas Food Corporation in Tanganyika. R. L. EDWARDS: Mushroom nutrition and fructification. R. L. EDWARDS and S. BURROWS: Bypaths in mushroom research.

20 October. Symposium on the plant and the law. W. C. MOORE: Legislation against plant diseases and pests in England and Wales. S. P. WILTSHIRE: Some repercussions arising from the exchange of plant material between countries. A. P. WINSOR: Inspection procedure in England. F. G. W. JONES: Sugar-beet eelworm Order. W. G. KEYWORTH: Verticillium wilt of hops Order. R. V. HARRIS: Some aspects of the certification schemes.

8 November. H. F. BARNES and Miss B. M. STOKES: Marking and breeding *Testacella* slugs. C. G. JOHNSON: Technical and biological aspects of insect drift. E. DUNN: Coloration of Colorado beetle wings. C. B. WILLIAMS: The relative efficiency of some insect traps. Miss G. WYKES: Preference of honey bees for sugars in nectars. J. H. WILSON: The expression of symptoms of leaf-roll virus in potatoes. Mrs S. S. WILLIAMS: The effect of microclimate on the establishment of timothy grass.

13 December. Symposium on some aspects of applied biology overseas. S. P. WILTSHIRE: Plant pathology in the colonies. F. W. ANDREWS: Some weed problems in the Sudan. H. OWEN: Cacao pod diseases in West Africa. W. J. HALL: Control and administration of entomological research work in the colonies. W. F. JEPSON: The work of the colonial government entomologist. A. H. STRICKLAND: The entomological problems associated with swollen shoot virus disease of cacao in West Africa. J. F. NEWMAN: The development of insecticide-resistant fly populations.

By kind permission of the Secretary of the Zoological Society of London and the Superintendent, about 50 members and guests attended the Field Meeting of the Association at Whipsnade Park on 19 May.

The Summer Meeting was held in Scotland during 4–7 July, with headquarters in Edinburgh; about 100 members and guests attended. On 4 July, following an Address of Welcome from Sir Edward Appleton, Principal of Edinburgh University, the following papers were read: K. W. BRAID: The relation of bracken control to hill-land farming. J. W. GREGOR: The wintering problem with special reference to cattle. J. A. CAMPBELL: The sheep tick problem in Scotland. A. FOGGIE: Tick-borne diseases of sheep in Scotland. D. O. MORGAN: Helminths in Scottish hill sheep. C. H. CADMAN: Recent work on raspberry virus diseases. R. D. READ: Breeding strawberries for disease resistance. T. P. MCINTOSH: A review of the main potato activities of the Department of Agriculture, 1918–50. W. BLACK: Potato breeding in relation to disease resistance. In the evening members and guests were entertained at a Civic Reception by the Lord Provost and Council at the City Chambers.

On 5 July visits were paid to the Seed Testing, Plant Registration and Plant Pathology Station; to the Scottish Plant Breeding Station; to the Scottish Seaweed Research Institute; to the University Department of Mycology and to the Royal Botanic Garden. During the evening a Conversazione was held in the University.

On 6 July one party visited Aberdeen and were conducted round King's College, the Macaulay Institute for Soil Research, the Rubislaw Granite Quarry, Craibstone Farm and the Rowett Research Institute. Another party visited the West of Scotland Agricultural College and the Hannah Dairy Research Institute, Auchincruive.

On 7 July a party toured the Trossachs and studied problems connected with bracken eradication and forestry.

The Report of a subcommittee appointed to consider the formation of a Scottish Group within the Association was accepted and adopted. The main conclusion reached was as follows: 'Taking the results of this enquiry into account, the subcommittee feel unable to recommend the formation of a separate Scottish or Northern Group, or indeed of *any* regional group. They are of the opinion that the interests and wishes of members living at a distance from London would best be met by holding at least one Provincial meeting annually, apart from the spring and summer meetings, and by paying particular attention to the needs of Scotland, Northern Ireland and Wales. They suggest that local arrangements for such meetings could best be left in the hands of members in the locality where the meeting is to be held, without the appointment of a separate Secretary for Scotland or any other region.'

Law XVII was amended to read:

'At a Council Meeting not less than one month prior to the Annual General Meeting, the Council shall nominate a President, a Treasurer, two Secretaries, an Editor and an Assistant Editor of the *Annals*. At least three weeks before this Council Meeting a notice shall be sent to all Members giving the names of the four retiring members of Council and asking for nominations, duly seconded, to fill the vacancies. Nominations received shall be included with any put forward by the Council. If the number of persons proposed for Council exceeds the number to be elected, the Secretary shall forthwith arrange a postal ballot among all members of the Association. The President shall nominate two members, not candidates for election, to act as Scrutineers of the ballot papers returned. The Scrutineers shall reject any ballot paper which supports candidates in excess of the number to be elected. They shall report the result of the ballot to the President before the Council Meeting immediately preceding the Annual General Meeting and in the event of an equality of votes between two or more candidates the President shall choose between them. The names of the Officers and new Council shall be read at the Annual General Meeting and circulated to all members with the notice of the next meeting.'

The Association was represented on the Parliamentary and Scientific Committee, and on the Biological Council. Two members served as Delegates to the 7th International Botanical Congress in Sweden.

The Council recorded with pleasure the honours conferred by the King on the following members during the year: Prof. J. W. Munro, C.B.E., Mr F. J. Chittenden, O.B.E., Dr T. Goodey, O.B.E., Dr T. P. McIntosh, O.B.E.

The Hon. Secretary, Dr Wood, was awarded a Commonwealth Scholarship, and was granted a year's leave from September. Dr Broadbent was nominated Acting Hon. Secretary in his absence. The Council wishes to place on record its grateful thanks to Dr Busvine who is resigning from the office of Hon. Assistant Secretary, which he has held for four years.

The Council wish to record their appreciation of the work done by Dr Foister and his colleagues in arranging the Summer Meeting.

The Council wish once again to thank the authorities of Imperial College for their kindness in providing lecture theatres for meetings, and for the use which has been made of their catering facilities.

REPORT OF THE HONORARY TREASURER FOR THE YEAR ENDING 31 DECEMBER 1950

During the year ended 31 December 1950, the amount received for subscriptions and entrance fees, including arrears, was £709. 4*s*. 6*d*., an increase of £54. 2*s*. 0*d*. This increase is partly accounted for by fewer members being in arrears at the end of the year.

Income from the sale of the current volume of the *Annals of Applied Biology* amounted to £1650. 8*s*. 3*d*., an increase of £61. 8*s*. 3*d*. over the sale of the *Annals* in 1949. The cost of production was £2591. 0*s*. 3*d*., an increase of £490. 3*s*. 3*d*. The income from the sale of back volumes and parts was £117. 1*s*. 3*d*., a decrease of £157. 14*s*. 9*d*., while the sale of reprints showed a decrease of £26. 5*s*. 8*d*.

Income for the year showed a deficit of £98. 1*s*. 10*d*. over the expenditure as against an excess of £537. 19*s*. 4*d*. in the previous year. This is accounted for by the greatly increased deficit on the *Annals*, and the increased expenditure on printing, stationery, postage and the cost of the summer meeting at Edinburgh.

The Association received a grant of £200 from the Royal Society in aid of the *Annals*. This grant is gratefully acknowledged.

The net assets of the Association at the end of the year amounted to £3143. 4*s*. 8*d*., an improvement of £101. 18*s*. 2*d*. as compared with a year ago. All available surplus funds are invested in the Post Office (£1291. 11*s*. 9*d*.), National Savings Certificates £1366. 13*s*. 3*d*.) and 2½% Defence Bonds (£400). The estimated value of the stock of the *Annals of Applied Biology* is £181. 10*s*. 0*d*.

It may be noted that the increase in membership fell to a more normal figure from the abnormally large increases of the immediate post-war years and the sale of back volumes and parts fell as predicted. The expected increase in the cost of production of the *Annals* was experienced and it can safely be assumed that the coming year will see a still further increase.

HON. EDITOR'S REPORT FOR 1950

Vol. **37** of *The Annals of Applied Biology*, with xii + 707 pages and 14 Plates, showed an increase of 135 pages over vol. **36**. Including papers published in *Proceedings* vol. **37** contained 61 communications (54 by members), the subjects being roughly classified as follows: general applied botany, 10; mycology and plant diseases, 10; viruses and virus diseases, 11; entomology and insect pests, 12; insecticides and fungicides, 9; plant nematodes, 5; general applied zoology, 1; statistical methods, 3.

The printing order for 1950 remained at 1450 copies.

The dates of issue of the four parts were: no. 1, 17 March; no. 2, 14 July; no. 3, 14 November; no. 4, 30 December. The late issue of no. 3 was due to delay in binding which occurred as an after-effect of the London printing dispute.

There are two resignations to record from the Publications Committee—Dr Hubert Martin, who has been a member of the Committee since 1945, and Mr D. J. Finney, who joined the Committee in 1946. Both have given the *Annals of Applied Biology* exceptional service, for which the Editors express their sincere appreciation.

ASSOCIATION OF APPLIED BIOLOGISTS PLANT PESTS AND DISEASES COMMITTEE, REPORT FOR 1950

Two meetings of the Committee have been held to date, on 29 September and 12 December. E. Dunn, E. C. Large, A. F. Posnette and W. H. Read were appointed by Council to fill the vacancies caused by retirement under the rules of S. H. Bennett, R. H. Gregory, Miss M. A. Keay and I. W. Selman. At the first meeting H. E. Croxall was elected Chairman and N. G. Morgan, Secretary.

The Sub-Committee appointed to prepare a list of terms requiring definition, used in Economic Entomology, has circulated a preliminary list to a number of entomologists.

A Sub-Committee has been formed to collect, sift and record information relating to susceptibility of fruit varieties to damage by foliage spray materials.

The establishment of crop observation plots on which the natural infestation by pests and diseases can be studied, is under consideration.

A second revision course in Plant Nematology is to be considered at the first meeting in 1951.

REVIEWS

Recent Advances in the Study of Plant Viruses. By K. M. Smith. Pp. viii + 300. London: J. and A. Churchill Ltd. 1951. 22*s.* 6*d.*

The general title 'Recent Advances' for a series of books may have been a good idea once, but the publishers should consider abandoning it. The first edition of this book was published in 1933 and was the first book written on the subject; the title was then accurate but unnecessary, because all 'advances' in the study of plant viruses were of necessity 'recent'. During the 1930's the subject developed with great rapidity and the contents of the first edition have now for many years belied its title. This has been fully recognized by the author who has produced an entirely new book for the second edition. The title is now misleading and inadequate, because the book does not deal only with recent work. It is a valuable account of the development of knowledge on plant viruses and virus diseases and, indeed, is least satisfactory when the most recent advances are being described.

The chapter headings will indicate the scope of the book: these are, (I) Introduction; (II) Symptomatology; (III) Physiology of Virus-Diseased Plants; (IV) Insects in Relation to Viruses; (V) Methods of Transmission of Plant Viruses; (VI) Methods of Purification of Viruses; (VII) Properties of Purified Viruses; (VIII) The Sizes of Viruses and the Methods Employed in their Estimation; (IX) Electron Microscopy of Plant Viruses; (X) Strains, Mutations and Variation in Viruses; (XI) The Serology of Plant Viruses; (XII) Control of Plant Virus Diseases: Classification and Nomenclature of Plant Viruses.

The chapters are not all of the same quality. Chapters II, IV and V deal with subjects to which the author himself has made many original contributions and are outstanding. Chapters VI and VII are neither adequate nor up-to-date. Chapter VIII is a reprinted article first published jointly by the author with Drs Lea and Markham in 1942, and it differs rather strikingly from the rest of the book. It deals mainly with things other than plant viruses, and is a detailed treatment of methods used for estimating particle sizes from measurements of physical properties. For the virus worker most of these methods have been rendered unnecessary by developments in electron microscopy, as is beautifully portrayed in chapter IX.

The fifty-one illustrations have been carefully chosen and are well reproduced. There is a subject and an author index, and the Bibliography is more than adequate. The book, despite some shortcomings and ambiguous phraseology, provides a valuable introduction to the study of plant viruses.

F. C. BAWDEN

Applied Mycology and Bacteriology. By L. D. Galloway. Pp. viii + 184. London: Leonard Hill Ltd. 3rd edition, 1950. 12*s.*

A detailed review of the first (1937) edition appeared in vol. 35, p. 438 of the *Annals of Applied Biology*. The rapid development in microbiology since that date is reflected in the numerous changes in the new edition. In Part 1, the sections on the classification of yeasts, bacteria and actinomycetes have been re-written, and the reference to viruses much extended. Additional material is included on the influence of environmental factors on the metabolism of fungi and bacteria. An introductory paragraph on photomicrography has been inserted.

In part 2, dealing with applications of microbiology, the outstanding changes are in the sections dealing with antibiotics and vitamins. A note has been added on the use of bacteria and fungi for vitamin assays.

In spite of these additions, Mr Galloway has, by judicious pruning of references of ephemeral value, retained the convenient size of the volume, while adding to its proved usefulness as an introduction to the field of industrial microbiology.

R. W. MARSH

Leaf Analysis. By H. LUNDEGÅRDH, translated by R. L. MITCHELL. Pp. vii + 176. London: Hilger and Watts. 1951. 22*s.* 6*d.*

Prof. Lundegårdh's monograph *Die Blattanalyse*, which was published by Gustav Fischer in Jena in 1945, was revised and extended by the author in 1946. An English translation of the 1946 edition, undertaken by Dr Mitchell of the Macaulay Institute for Soil Research, Aberdeen, was submitted to press in 1948 and now appears after 3 years delay. As a result of Dr Mitchell's painstaking work, Lundegårdh's results, collated over a period of 20 years at the Plant Physiology Institute, Uppsala, become available to a wider public.

The title, 'Leaf Analysis', is somewhat misleading, as the book deals only with results for nitrogen, phosphorus, potassium, calcium and sodium. Micronutrient elements, which are of considerable importance in plant growth, are not discussed, with the exception of manganese. Moreover, almost all the analyses reported are confined to cereal crops and pasture grasses.

The book is divided into three chapters. The first, dealing with fundamental problems of salt absorption by higher plants, and of nutrient antagonisms in soil, shows that complex physico-chemical and biological relations in soils preclude the use of soil analysis alone in studies of nutrient uptake and requirements of plants. In the second chapter, devoted to the practical basis of leaf analysis, Lundegårdh gives details of his methods including the now well-known techniques of spectrochemical analysis developed at Uppsala. Numerous illustrations are given of the close relationship between yields of grain in cereals and Lundegårdh's '*S*' values for nitrogen, phosphorus, potassium and calcium (mg. element, divided by its atomic weight, in 100 g. dry matter of plant). The last chapter is concerned with the application of leaf analysis to the study of manurial requirements of soils, with particular reference to cereals and pasture grasses. The author convincingly shows that leaf analysis for nitrogen, phosphorus, potassium and calcium is more valuable than the soil data for these elements in determining the fertilizer treatment required for maximum yields of grain.

The choice of '*S*' values for expressing the results of plant analysis emphasizes the importance of securing an agreed method of presenting such data. The results presented in some tables, e.g. nos. 35 and 36, tend to be unwieldy and could with advantage be reduced to the statistical analysis of the factorial experiments.

The translation, which has clearly necessitated much thought and labour, is generally satisfactory. In some instances the insistence on conforming exactly to the original text introduces a German rather than an English phraseology, and there is an occasional mixing of tenses.

The book contains a wealth of data and should be of great interest to plant physiologists, agronomists and all concerned with evaluating fertilizer responses of crop plants. Soil chemists should note Lundegårdh's indisputable evidence on the importance of supplementing soil data with analyses of the crop plants when studying the manurial requirements of plants and soils.

D. J. D. NICHOLAS

A Synopsis of the World's Literature on the Fruit Tree Red Spider Mite (Metatetranychus ulmi) (*C. L. Koch*, 1838) *and its Predators.* By JOAN R. GROVES, with a review of the problem by A. M. MASSEE. Pp. 180. London: Commonwealth Institute of Entomology. 1951. 20*s.*

Miss J. R. Groves's valuable and monumental synopsis of the world's literature on the red spider is a testimony to the importance of a pest created by man's efforts to produce better and more fruit. The failure to control the pest has become a classic example of the applied entomologist's struggle to circumvent the adverse biological consequences of chemical control measures.

In the introduction, Dr A. M. Massee, an authority who has made so many valuable contributions to our knowledge of the mite, has given in four pages an excellent and up-to-date

review of the problem. He points out that the natural control of the mites by predators is of considerable importance. This presents the entomologist with a major problem in that all of the highly potent new insecticides with prolonged residual toxicities may result in devastating spider infestations. Even if an efficient summer ovicide is found, the problem is far from solved, as the complete coverage by a spray of the under surface of all the leaves is practically beyond economic achievement. It would therefore seem that the most likely solutions to the problem are the discovery of a cheap and highly efficient winter ovicide or a foliage systemic poison. Let us hope it is not a systemic.

Miss Groves must be congratulated on the care she has used in her exhaustive survey of the literature: in fact it is impossible to find any notable omission. Each reference has a short, brief and accurate summary. The author's patience must have been sorely tried by the monotonous repetition of the similarity of the conclusions reached by investigators all over the world, combined with the sparsity of critical work; a state of affairs caused by the extreme difficulty of working with red spider under laboratory and field conditions.

Until a simple and highly effective control of 'spider' is discovered the greatest care must be exercised in the use of the organic insecticides, as some of these almost completely eliminate insect life from an orchard but leave red spider to ruin the crop.

Fruit growers and entomologists are indebted to Miss Groves and Dr Massee for crystallizing our knowledge of an important pest. H. G. H. KEARNS

Selected Invertebrate Types. Edited by F. A. BROWN, Jnr. Pp. 597. New York: J. Wiley and Sons, Inc.; London: Messrs Chapman and Hall. 48*s*.

This book is a guide to the practical study of certain invertebrate organisms. Its text is not limited to the bare essentials necessary for practical work but gives much general information on the types described. In addition to its main function as a laboratory manual for the undergraduate, the thirteen contributors hope that the book may introduce '...investigators to species they might profitably select for their own experimental investigations'.

Whilst the text is of a very high order the book is not without faults. From his earliest practical work the student should learn to record his results in clear, well-annotated drawings with short descriptive notes. A practical guide should do all in its power to create the atmosphere of a first-hand search and by example should show the student the value of records which really refer to the particular specimen being studied. A good number of the figures should be dissection-like and helpful, rather than diagrams or reproductions of figures already available in the usual text-books. 'Selected Invertebrate Types' sets a poor example as far as figures are concerned. In spite of the statement in the preface that 'many figures are original and drawn from the actual specimens...'; too many are modifications of other people's work. Such phrases as 'Drawn after...', 'Compiled and modified after...', 'Compounded from publications of several authors...', occur too frequently for a laboratory manual and rarely is a scale incorporated in the figures. Furthermore, the distribution of illustrations lacks balance. For instance, some sixty pages of text, without illustrations, are devoted to *Limulus* and the Decapod Crustacea. In view of their complexity and the general usefulness of such organisms, for physiological as well as anatomical studies, some drawings should have been included.

The choice of organisms detracts from the general usefulness of the book. Out of 125 types, 34 are Protozoa, the majority of which can be found figured in the usual text-books. Insects suffer their usual neglect being represented by *Periplaneta* (with some brief remarks on *Blaberus craniifer*) and the larva of *Drosophila*; these types are however very admirably described and this section is one of the best in the book. Even Crustacea are unable to boast of favouritism, for their total only musters *Artemia*, *Daphnia*, *Cyclops*, *Lepas*, *Heteromysis* and *Callinectes*. With such a choice of well-known organisms it is a little difficult to believe that 'the investigators' are going to find new experimental animals.

The 26-page bibliography arranged according to types is certainly useful. Its contents, however, show that some of the authors are not up-to-date with the organisms which they have described. For instance, the anatomy of *Arenicola cristata* is described, the figures being based on Ashworth's well-known L.M.B.C. memoir (1904) on *Arenicola marina*. Four references on *Arenicola* are cited, the most recent being 1939, but no mention is made of the very stimulating investigations of G. P. Wells, the first paper of which was published in 1937.

If the reviewer has concentrated on the deficiencies of this book it is not without reason, for one of the responsibilities of a University teacher is to recommend text-books to students and the reviewer cannot bring himself to give whole-hearted support to this publication. It will form a useful additional reference book but it falls short of the book that it aims to be and might even become, in a later edition. J. B. CRAGG

Gall Midges of Economic Importance. Vol. VI. *Miscellaneous Crops.* By H. F. BARNES. Pp. 229. Agricultural and Horticultural Handbooks. London: Crosby, Lockwood and Son Ltd. 1949. 15*s.*

Of Dr Barnes's eight projected volumes on Gall Midges, this is the fifth to be published. It was inevitable that one volume should deal with 'Miscellaneous Crops'. These comprise bamboos, basket willows, beverage plants, dye plants, fibre or textile plants, herbs (aromatic, culinary and medicinal), insecticide plants, oil plants, rubber plants, spice and sugar plants. Each section starts with Dr Barnes's well-known and very useful introductory notes on the uses for which the plants are grown, and a list of the crops, the midges attacking them and the parts of the plants attacked.

The volume fully maintains the standard of the preceding volumes; in particular, the indexing is again so good that it calls for special comment; there is an index of the generic, specific and popular names of the midges mentioned, a plant index and a general index: there is a list of 362 references in addition to a list of the most important references given at the end of each section.

It is again emphasized that the author has dealt with the gall midges of the world and that he is a leading authority on this group of insects. This being so, it is inevitable that entomologists the world over will from time to time need to use these books. When their value is fully realized abroad there will almost certainly be a sudden increased demand and intending purchasers will do well to bear this in mind. I. THOMAS

Cell Growth and Cell Function. By T. O. CASPERSSON. Pp. 185. New York: W. W. Norton and Co. Ltd.; London: Chapman and Hall Ltd. 1950. 28*s.*

The title of this book is misleading. The sub-title, 'A Cytochemical Study', is more appropriate, but even so suggests a wider subject than is actually covered. The text, which is less than 25,000 words, is based on the Salmon Memorial Lectures given by the author to the New York Academy of Medicine in 1948, and deals only with work done at the Karolinska Institute in Sweden. The 105 references in the Bibliography seem all to be publications from the Institute.

The book is largely an account of ultra-violet microspectrophotometry and will be invaluable to those wishing to use this technique. The results obtained by applying the technique to some selected types of biological systems are also described; in reading these sections it is important to remember that the interpretations depend on the assumption that absorption is specific to the substances postulated.

The book is a lavish production and more than half of the space is occupied by the ninety-four figures. Some of these are beautiful photomicrographs, but many are line drawing or monotonously similar absorption curves. At least three figures are reproduced twice. Fig. 91

occupies the whole of p. 173 and is identical with Fig. 94 which occupies p. 177. These figures purport to illustrate virus reproduction, but contain little that is both novel and likely to be true. At least one might have been omitted in favour of including an Index. F. C. BAWDEN

Physiology of Fungi. By LILIAN E. HAWKER. Pp. xvi + 360. London: University of London Press. 1950. 21*s*.

The importance of fungi is being increasingly recognized. As plant pathogens they are now as significant as ever and may become even more so with intensification of agricultural production, particularly in backward areas. The production of fungicides for crop protection is now a large industry in all advanced countries, and for many years fungi have been used for the commercial production of certain chemicals. More recently a new and important industry has developed around the production of antibiotics for use in human and veterinary medicine. A vast literature covers the part played by these organisms in the production of food and beverages. Academically, by reason of special features of their sexual reproduction, certain species have become important tools in physiological genetics, and although the study of fungal metabolism has hardly begun, the scope of the field to be explored may be appreciated when one considers the divers substrates and products of their growth. In view of these and other facts it is surprising that in many courses leading to a first degree in Botany or related subjects the fungi are still treated mainly from morphological and taxonomic stand-points. There seems little doubt that too frequently little attention is paid to their biological activities except possibly as plant pathogens where, however, the anatomical features of the host-parasite relationship are likely to be the aspects most emphasized. This may in part have been due to the fact that while suitable texts on the structure and development of fungi are available there has, until recently, been none treating adequately their physiology and related phenomena. There can be few better qualified than Dr Hawker to write a book meeting this deficiency. For some years she has taught systematic mycology, fungal physiology and plant pathology to undergraduate and graduate students, and at the same time been an active worker in the widely separated fields of vitamin requirements of fungi and diseases of gladioli and narcissi.

The scope of her work is wider than the title suggests, as it includes sections on subjects which would not normally be considered physiological, e.g. relations between fungi and insects. The book is divided into eight chapters, each of which is fairly self-contained. The first is introductory, giving descriptions in general terms of the vegetative and reproductive structures found among fungi. The treatment will be useful in refreshing the memory of those who have been out of contact with mycology for some time, but in the absence of further background cannot be expected to be adequate for full appreciation of what follows later. As with higher plants a detailed knowledge of the morphology and anatomy of the organism should generally precede investigations of its physiology. Chapter 2 considers methods used in culturing fungi and in measuring their growth. No reference is made, however, to the use of replacement cultures as an important technique in determining metabolic changes independently of assimilatory reactions. Growth forms and variation are then discussed in some detail. There follows a comprehensive account of nutrition with particular emphasis on accessory growth-factor requirements. Chapter 4 deals with respiration, fermentation and metabolic products and completes the general treatment of basal metabolism.

The effects of nutrition on sporulation are then treated in some detail, nutrient concentration, sources of carbon, nitrogen, other elements and accessory growth factors being considered in turn. This is followed in Chapter 6 by an account of the influence of factors other than nutrition on growth and sporulation. There is comparatively little given on the effects of pH and poisons are dealt with in less than two pages. The latter is surprising in view of the importance of fungicides and the extensive literature which has accumulated on their assessment and mode of action. Chapter 7 deals with factors influencing the survival and

germination of spores, and the final chapter is an account of the interaction between fungi and other organisms. As such, it necessarily groups together subjects having little or no relationship with one another. The processes involved in parasitism of higher plants by facultative and obligate pathogens are first described in some detail. This is succeeded by a description of mycorrhizae and of the association of fungi with algae. The interactions with fungi and bacteria are dealt with somewhat briefly when one considers the importance of antibiotic production and the increasing interest in the significance of associative effects in the establishment of disease under natural conditions. The chapter concludes with a survey of the relationships between fungi and animals.

Slightly distracting is the frequency with which reference is made to 'unpublished experiments by the author', particularly when the information is as interesting as that on p. 196 where the fructification of *Collybia velutipes* is described as behaving as an *Avena* coleoptile!

Each chapter is followed by a short bibliography and there is in addition a comprehensive list of over 800 references.

Dr Hawker approaches her subject as a biologist. This is particularly welcome in view of the increasing tendency to use fungi merely as tools in biochemical investigations which neglect studies of their functioning as organisms. Her book is a most useful contribution to mycological literature and will be welcomed by student and teacher alike. R. K. S. WOOD

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY*, INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1950 *Cr.*

Expenditure	£ s. d.	Income	£ s. d.
To Estimated Value of Stock at 1 January 1950	153 3 0	By Sales—Current Volume	1650 8 3
To Cambridge University Press	2591 0 3	By Sales—Back Volumes, Parts and Sets	117 1 3
		By Sales of Reprints and Advertisement	122 5 9
		By Estimated Value of Stock at 31 December 1950	181 10 0
		By Balance carried down	672 18 0
	£2744 3 3		£2744 3 3

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1950 *Cr.*

Expenditure	£ s. d.	Income	£ s. d.	£ s. d.
To *Annals of Applied Biology* balance brought down	672 18 0	By Members Subscriptions:		
To Printing and Stationery	71 3 4	Arrears	29 5 6	
To Postage and Cheque stamps	40 6 1	Entrance Fees	21 16 6	
To Subscription—Parliamentary Science Committee	7 7 0	Current	658 2 6	
To Donation—The Biological Council	5 0 0			709 4 6
To Donation—Institute of Biology	5 5 0	By Sundry Income—Spring Meeting		13 15 0
To Sundry Out-of-Pocket expenses of Editors, Secretaries and Treasurer	14 6 10	By Net Receipts—*List of Common Names*		2 2 0
To Refreshments	25 4 9	By Interest Received and Receivable:		
To Audit Fee	7 7 0	2½ % Defence Bonds	8 3 6	
To Spring Meeting	18 0 6	National Savings Certificates	28 7 2	
To Summer Meeting	24 5 6	Post Office Savings Bank	31 10 0	
				68 0 8
		By Balance, being Excess of Expenditure over Income for the year		98 1 10
	£891 4 0			£891 4 0

BALANCE SHEET, 31 DECEMBER 1950

Liabilities and Surplus	£ s. d.	£ s. d.	Assets	£ s. d.	£ s. d.
Sundry Creditors:			Cash:		
Cambridge University Press	751 5 0		Westminster Bank: Current Account	672 6 4	
Audit Fee	7 7 0		In hands of Treasurer	202 10 0	
Sundry Expenses	4 11 8		Post Office Savings Bank Account	1291 11 9	
		763 3 8			2166 8 1
Subscriptions and Entrance Fees paid in advance		33 3 0	1500 National Savings Certificates (Cost £890. 1*s.* 0*d.*)		1366 13 3
Life Membership Fund Reserve		175 0 0	2½ % Defence Bonds		400 0 0
Excess of Assets over Liabilities:			Stock of *Annals of Applied Biology* as estimated		181 10 0
As per Balance Sheet 31 December 1950	3041 6 6				
Add: Royal Society Grant	200 0 0				
	£3241 6 6				
Less: Balance of Income and Expenditure Account for 1950	98 1 10				
		3143 4 8			
		£4114 11 4			£4114 11 4

H. F. BARNES, *Hon. Treasurer*

I certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to me, and in my opinion, the Balance Sheet exhibits a true and correct view of the state of affairs of the Association according to such books, vouchers and documents.

(Signed) J. B. BENNETT *Chartered Accountant* } *Auditor*

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